Comments from the virtual meeting:

In general comments were supportive and editors felt this was a live topic and a well performed study, this was based on fairly supportive reviews.

We thank the Editorial Committee and Reviewers for their positive feedback on our manuscript.

Many of the reviewers had concerns about the novelty of this submission, and this was discussed again at our meeting. However, it was an editorial decision that this paper did add sufficiently and was still a very important issue given the different results out there. But it would be good if the authors could address some the issues raised about novelty by the reviewers.

We thank the Editorial Board for the opportunity to clarify the novelty of our study. Our motivation for conducting this study was primarily based on the apparent contradictory results of the recently published studies of pioglitazone and the risk of bladder cancer. Given the time that has elapsed since the initial concerns regarding this potential adverse drug reaction, these recent studies had longer follow-up than earlier ones. For example, in the interim 5-year analysis of the Kaiser Permanente study, the use of pioglitazone was associated with a 40% increased risk of bladder cancer with at least 2 years of use (Lewis et al. Diabetes Care 2011). However, in the final analysis, which was based on the same cohort using similar analytical methods, the use of pioglitazone was no longer associated with an increased risk of bladder cancer after a 10-year follow-up (Lewis et al. JAMA 2015). Thus, to assess whether the association is "attenuated" with time, we conducted our study using a cohort of that followed patients for up to 14.5 years of follow-up. In contrast to the recent studies, our findings suggest that the risk of bladder cancer does not attenuate with time. As explained in the Discussion section of the manuscript, we believe the discrepancy between our findings and those of these recent studies is likely due to important methodological shortcomings in these studies.

To further emphasize the point above, we clarified the need for additional studies with longer follow-up (and hence the novelty of our study) in the Introduction section of the revised manuscript (Page 4).

There was also a discussion following the reviewers' comments about the lack of information about how important confounders were handled, and several of the editors had similar concerns. However, it was felt that many of the reviewers' comments were addressable.

We thank the Editorial Committee and Reviewers for raising this issue. We now provide a detailed description of how the confounders were defined and handled in the analyses (Page 8).

We also took this opportunity to conduct an additional sensitivity analysis using multiple imputation for variables with missing information (i.e., body mass index, smoking, and

haemoglobin A1c). This analysis led to consistent results. This information can be found in the Methods section (Page 9, second paragraph) and in Figure 2.

It was an editorial decision that the concerns raised about ethics was not a priority given there is mention a statement from the Clinical Practice Research Datalink committee in the UK and a vote from Canada.

All data from the Clinical Practice Research Datalink (CPRD) are anonymized for research purposes, and all protocols must obtain scientific and ethics approval from the Independent Scientific Advisory Committee (ISAC) of the CPRD. As noted by the Editorial Committee, our protocol underwent further approval by the Research Ethics Board of our institution. As such, we agree with the Editorial Committee that the ethical issues raised by the Reviewer are not a concern.

First, please revise your paper to respond to all of the comments by the reviewers. Their reports are available at the end of this letter, below.

Please also respond to these additional comments by the committee:

1. *Please clarify what is meant by misclassification being possible because of CPRD recording prescriptions written by GP's and not those filled or taken by patients. The GPs on the editorial board felt that pioglitazone is not something they would start the patient on since it is a niche drug (but rather an Endocrinologist would do), but they are likely to prescribe the refills.

In the limitations section of our manuscript, we acknowledged that the possibility that exposure misclassification was possible if some patients received pioglitazone exclusively from a specialist (such as an endocrinologist). However, we agree with the Editorial Committee that in most instances, that the general practitioner would be responsible for prescribing refills. This is now clarified in the revised manuscript (Page 16, first paragraph).

2. *We would particularly like you to respond and address the points made by the reviewer Bell

We have carefully considered the points made by all reviewers, paying particular attention to those raised by Dr Bell. Our response to her comments can be found below.

3 * There were mixed reviews about confounders and we'd like some more clarification about how they were handled. Specifically our statistician's main concern is the use of time varying Cox models. In this model only the main exposure was updated but not the covariates. This may have been done because of concerns of inappropriate control of "time varying" confounders, which is a relevant concern. However, here, it remains the question whether confounding is adequately addressed.

The Editorial Committee raised an important point. Indeed, while our primary analysis considered exposure as a time-varying variable, the confounders were measured at cohort entry. As such, we acknowledge that this approach may be subject to some residual

confounding. We had considered including time-varying confounders but, as pointed out by the Editorial Committee, we were concerned about inappropriately adjusting for variables found on the causal pathway (and thus adjusting for the consequences of exposure).

To further address this issue, we conducted an additional sensitivity analysis using a marginal structural model using inverse of probability of treatment weights (IPTWs). This approach deals with potential confounders that can be also be in the causal pathway between the exposure and outcome (Hernán et al. Epidemiology 2000;11(5):561-70; Platt et al. Eur J Epidemiol 2012;27(2):77-83). In addition, we also used inverse probability of censoring weights (IPCWs) to account for censoring (Cole & Hernan. Am J Epidemiol 2008;168(6):656-64). Reassuringly, this method led to results that are consistent with those of the primary analysis. We believe that the addition of this analysis greatly strengthened the manuscript.

The marginal structural model is now mentioned as an additional sensitivity analysis in the Methods section (Pages 9, bottom of the page), and a detailed description of how it was implemented is provided in the Supplemental file (Supplemental Methods in the Supplemental file). The results are provided in Figure 2, along with the other sensitivity analyses.

In your response please provide, point by point, your replies to the comments made by the reviewers and the editors, explaining how you have dealt with them in the paper.

Comments from the external peer reviewers

Reviewer 1 (Jim Catto)

Pioglitazone use and bladder cancer risk: a population-based cohort study by Tuccori et al.

The authors look to clarify uncertainty about the relationship and strength of association between pioglitazone use and bladder cancer. This follows several large reports (n=193,099 [20]) of conflicting data regarding this issue. In this work, the authors do find a link between the drug and subsequent bladder cancer (HR: 1.63, 95% CI: 1.22 to 2.19). They compare the findings with those for rosiglitazone. The large size/follow up allows a relevant sensitivity analysis that further supports the direct association.

The strengths of this report are the design (large cohort, clear inclusion criteria (newly treated), homogenous population and follow up rates). Weaknesses include the lack of granularity of the CPRD dataset and lack of novelty of the topic. For example, the authors adjust for many confounders in their model, but miss many that are relevant for bladder cancer aetiology.

1. Specific missing data include occupation (page 8 lines 6-25; as the second commonest cause of bladder cancer), and pelvic radiotherapy.

Occupational exposure and pelvic radiotherapy are known risk factor for bladder cancer. Unfortunately, these variables are not well recorded in the CPRD, and consequently, it was not possible to adjust for them in the models. This is now acknowledged in the limitations section of the revised manuscript (Page 15, last paragraph). However, it is important to note that while these variables are associated with the outcome, it is not clear whether they are associated with the exposure, as occupation and pelvic radiation are unlikely to be considerations for prescribing thiazolidinediones, a requirement for confounding.

Overall this work is a welcome addition to this field, but I wonder if it is enough to overturn the pre-existing conflicts. Certainly the sensitivity analyses are very convincing.

We thank the Reviewer for the positive comments.

2. How was smoking data handled? A binary Yes/No will be insufficient in this analysis

We agree with the Reviewer about the importance of properly quantifying smoking data. Unfortunately, the granularity of these data in the CPRD is limited, and hence it was only possible to consider it as a three-level variable (i.e., ever, never, unknown). Despite this limited granularity, we believe that the inclusion of this variable in our models remains a strength of our study, as smoking information is rarely collected in other settings, such as in administrative databases. Finally, as mentioned above, we conducted an additional sensitivity analysis using multiple imputation for variables with missing information, which included smoking, and observed consistent results (Figure 2).

3. Please explain "Pioglitazone users were …more likely to have prior bladder conditions" (page 11 and table 1). This is a concern (to my mind), as many patients with bladder cancer (esp. CIS) are misdiagnosed in the community with benign bladder conditions. To explore this, please look at the time from entry to bladder cancer diagnosis in patients with & without prior bladder conditions. Is there a difference? If the cancer diagnosis occurs within 3 years of entry – then I suspect these patients had occult malignancy at entry. Would the 2.9% difference in the rate of prior symptoms explain your findings? I do note no difference between the pioglitazone and rosiglitazone population, in this respect (sup table 5), but this is a smaller sample size.

The Reviewer raises an important point. Indeed, we observed a higher prevalence of bladder conditions in both pioglitazone and rosiglitazone users. This may reflect the fact that these drugs are prescribed to patients with more advanced disease. For this reason, we adjusted for the presence of such conditions at cohort entry.

To further address the comment raised by the Reviewer, we conducted an additional sensitivity analysis by excluding patients with a history of bladder conditions at any time prior to cohort entry and censoring those with new diagnoses during follow-up. The results of this sensitivity analysis were consistent with those of our primary analysis (pioglitazone, HR: 1.73, 95% CI: 1.27 to 2.35 and rosiglitazone, HR: 1.16, 95% CI: 0.85 to 1.58). This additional sensitivity analysis is now described in the Methods section of the manuscript (Page 9, second paragraph), and presented in Figure 2 and Supplementary Tables 3 and 4.

4. With concerns to misclassification of cancer (page 15, lines 8-15) – could you cross the CPRD with relevant UK cancer registries?

Unfortunately, it was not feasible to link our cohort with the UK National Cancer Data Repository. However, a previous study reported excellent concordance for cancers of the urinary tract between the CPRD and the UK National Cancer Data Repository (NCDR) using a cohort of patients with type 2 diabetes (i.e. out of the 424 events identified in the NCDR, 401 were recorded in the CPRD; Boggon et al., Pharmacoepidemiol Drug saf 2013;22:168-175). Furthermore, our overall incidence rate of bladder cancer (90/100,000 person-years) is consistent with what would have been expected in a cohort of patients with type 2 diabetes. Thus, while misclassification of the outcome is possible, we believe it was minimal in our cohort.

5. Please detail the time between starting an anti-diabetic agent and the bladder cancer diagnosis in all cohorts. This is not clear from your data.

We thank the Reviewer for this comment. The median (interquartile range) time between initiation of an antidiabetic drug (cohort entry) and a diagnosis of bladder cancer was 4.4 (2.5-6.5) years. This is now mentioned in the Results section (Page 12, first paragraph).

Reviewer 2 (Trevor Benn)

Originality
Although the authors cite previous work suggesting a risk of bladder cancer in patients taking a certain anti-diabetic drug, this is a major study which adds value to what was previously known.

Importance
Since diabetes is a widespread and potentially fatal disease, this study is important since it investigates a cancer risk associated with the diabetes treatment.

Scientific reliability
The study appears to be very reliable since it uses standard methodology, with a very large number of patients and a large number of cancer cases, and has obtained statistically significant results.

Overall design
Excellent - see comments in the previous paragraph.

Participants studied
The participants are appropriate to the research question.

We thank the Reviewer for the positive comments above.

Methods - including ethics
1. I think the methods are absolutely fine, but I have some concerns about the ethics. If the patients studied are UK residents there needs to be ethical approval from a UK

research ethics committee (REC) approved by the Health Research Authority (HRA) - approval by the ethics committee of a Montreal hospital is not sufficient, nor is one by an advisory committee for the database used (CPRD).

Were any identifiable data sent to Canada? The HRA requires that REC approval be given for any export of identifiable data outside the European Union.
I don't see any mention of how many patients were excluded because they did not consent to inclusion, which prompts the question of whether they were asked for consent. Under UK law the use of patient data without consent may be not just unethical but actually illegal if it is in breach of a legal duty of confidentiality. There is a way round this if a regulatory body (Confidentiality Advisory Group or Health and Social Care Information Centre) gives permission for research to be carried out without participants' consent, so we need to know whether such consent was obtained.

It may be that the custodians of the database have obtained a general ethical approval covering not only the present study but also any others using the database. If this is the case then the manuscript should say so, and give the name of the approving REC. It may be that the database only includes patients who have consented (on either an opt-in or opt-out basis) so that no legal exemption for patient consent is necessary. Again the MS should say if this is the case.

In short, the paper needs to give enough information to provide assurance of ethical approval and legality for the present study, which will also remind readers, if they need reminding, of the need to careful of such matters in their own research.

We thank the Reviewer for the opportunity to clarify this issue. As with all CPRD studies, our study protocol required approval from the Independent Scientific Advisory Committee (ISAC) of the CPRD to assess its scientific and ethic integrity. The ISAC-approved protocol was also approved by the Research Ethics Board of our institution (Lady Davis Institute, Jewish General Hospital, Montreal, Canada).

All the patient data used for our study were anonymized by the CPRD, and we adhered to all ISAC rules regarding the reporting of the results, such as suppressing cells with less than 5 patients (see for instance Supplementary Table 1). Given the anonymized nature of the data provided by the CPRD, no patients were contacted regarding our study but all consented to the use of their information for research purposes.

Results

The results of the study are clearly set out.

Interpretation and conclusions
The conclusion that one of the drugs studied has a causative link to bladder cancer appears to be well justified.

Abstract
This is a fair summary of the study and its conclusions.

We thank the Reviewer for his supportive comments above.

Reviewer 3 (Ian Clements)

Review of "Pioglitazone use and bladder cancer risk: a population based cohort study"
Dr Ian Clements 19th November, 2015

This paper is of immense importance to patients who have diabetes type 2 and are presented with the option of being treated with either pioglitazone or rosiglitazone to control this co-morbidity. Earlier studies have found conflicting outcomes regarding the former increasing the risk of bladder cancer; this has caused concern amongst both doctors and the patients (some of whom I believe have sued on the basis of their bladder cancer). These disparate results may have been because of smaller numbers, shorter time-spans, no comparison drug, or poor research design. This paper notes this, and addresses the issues by having many more patients than those earlier studies, and covering a longer time-span – over 15 years.

We thank the Reviewer for the positive comments.

As well as determining whether pioglitazone increased the risk of bladder cancer, they were also able to make a meaningful comparison with the similar (of the same class) aforementioned alternative – to the latter's advantage.

The authors seem to have addressed most, if not all, of the significant confounding factors and explained why some missed would probably be an insignificant influence on their conclusions: that pioglitazone use increases the risk of bladder cancer. This effectively resolves the uncertainty that resulted from the conflicting results of the earlier studies and will be a welcome piece of information for all type 2 diabetic patients who may be offered this drug.

1. On the issue of areas relevant to patients that are missing seems to be the researchers purposefully excluding patients from all stages of the design and, most oddly to me, "There are no plans to involve patients in the dissemination of results." There is no explanation for this, other than the implicit one that none in the intended audience would find it odd. The implication is that only doctors would know of these results, and then only if they chance to read about this research and then may tell their patients – this appears contrary to patients being able to give fully informed consent to the usage of pioglitazone. Whilst I appreciate that this research may well need to be filtered via the regulatory regimes such as NICE in the UK, and, if the results are accepted by them, perhaps made mandatory (that this drug not be used); but until then, patients are put at unnecessary risk and may successfully sue. I suggest that given the wide applicability of the conclusions – that use of pioglitazone increases the risk of bladder cancer and that this is a drug that may be offered to any diabetic patient – then perhaps the authors ought to have advocated wide publicity.
The challenge that patients face as a result of this, and all too much medical research, is knowing about it before they are called upon to make a fully informed decision. Given that there is at least one alternative drug which appears as efficacious, this is a pity.

We thank the Reviewer for his valuable comments and the opportunity to clarify our dissemination plans. While we do not plan on having patients participate as disseminators of our results, we fully agree with the Reviewer that patients need to be aware of this potential association. For this reason, they represent a key group of potential knowledge users that will be targeted by our knowledge translation and dissemination plans, which will include a press release issued by the Public Relations Department of our institution. This press release will disseminate results to key stakeholders including regulatory agencies, guidelines writing committees, practicing clinicians, and patients to provide them with the information required for informed decision making regarding the potential benefits and risks of pioglitazone use.

2. The only reference omissions I found were two meta-analyses "Assessing the Association of Pioglitazone Use and Bladder Cancer Through Drug Adverse Event Reporting" and "Pioglitazone and risk of bladder cancer: a meta-analysis of controlled studies" Both of which supported this study's conclusion.

In addition to the 2 articles cited above (notably, the first one is not a meta-analysis but a pharmacovigilance study), there are other meta-analyses of this association (i.e., Turner et al., Br J Clin Pharmacol. 2014 Aug;78(2):258-73; He et al., Tumour Biol. 2014 Mar;35(3):2095-102; Monami et al., Acta Diabetol. 2014 Feb;51(1):91-101) However, we decided not to cite meta-analyses of observational studies because we believe many of the studies included in these meta-analyses have important methodological shortcomings. The pooling such studies can be problematic, producing precise but biased results. Instead, we have cited all of the individual observational studies assessing the association between pioglitazone and bladder cancer, discussing their methodological shortcomings where appropriate.

Reviewer 4 (Francesco Trotta)

The article from Tuccori et al. contributes to address an important issue concerning the risk of bladder cancer following pioglitazone use. This is a very important topic since many observational studies have been conducted during last few years, however with controversial results on the risk estimates for bladder cancer (as also acknowledged by the authors), both for methodological limitations or for residual confounding.

We thank the Reviewer for his positive comments.

The present study run in the UK setting is conducted also with the attempt to overcome methodological limitations such as the "disease latency" and the drug exposure as time-varying variable. However, some subgroup analyses remain to be performed to further challenge the robustness of results and to increase the data reliability.

Specific issues are following:

1. Firstly, risk estimates of bladder cancer should be presented through a specific analysis by the three subgroup populations: i) incident users of antidiabetics; ii) switchers; iii) add-on therapy.

We are unclear about the rationale behind stratifying the cohort into the aforementioned 3 groups. It is important to note that since pioglitazone and rosiglitazone are second- to third-line treatments, the use of these drugs should be used at a similar stage in the disease process in all the 3 groups listed above. To further investigate this issue, we conducted an analysis stratifying the cohort into new users (i.e., newly-treated) and add-on/switchers. We obtained nearly identical results in both sub-cohorts for pioglitazone (new users: HR: 1.63, 95% CI: 1.17 to 2.25; add-on/switchers: HR: 1.63, 95% CI: 0.85 to 3.13) and rosiglitazone (new users: HR: 1.08, 95% CI: 0.76 to 1.52; add-on/switchers: HR: 1.09, 95% CI: 0.62 to 1.91).

2. In addition, a description of the ascertainment of use for each antidiabetics during the follow up (in the two periods, i.e. in the 1-year latency period and in the post latency period) should be provided in full (also as an adherence measure e.g. MPR if possible). Details on definition of exposure over time should be provided. To be clear: for example, an incident user of pioglitazone should have filled how many prescriptions per month (or per quarter or per year) to be considered on treatment? Similarly a definition of switchers should be included in the methods.

As described in the revised manuscript, patients were considered exposed to an antidiabetic drug during follow-up starting one year after a first prescription (i.e., after considering a one year lag period for latency purposes). We agree with the Reviewer that perhaps one prescription may not be enough to consider a patient exposed to pioglitazone or rosiglitazone. While the cumulative duration and dose analyses included as secondary analyses should quell such concerns, we conducted an additional sensitivity analysis using a stricter exposure definition. Specifically, to be considered exposed to pioglitazone or rosiglitazone, patients were required to have received at least 4 prescriptions within a 12-month moving window. Patients were considered exposed starting the year following the fourth prescription. The results of this sensitivity analysis led to a higher HR for pioglitazone (HR: 1.76, 95% CI: 1.29 to 2.39) and a lower HR for rosiglitazone (HR: 1.01, 95% CI: 0.73 to 1.39).

The description of this new sensitivity analysis can be found in the Methods section and the results are presented in Figure 2 and Supplementary Tables 3 and 4.

3. Secondly, a subgroup analysis between the two groups (pioglitazone and no-TZD users) to show how risk estimates varies according to a series of variables, also considered as confounders, should be included. Specifically, the effect of age, BMI, smoking, HbA1C, duration of treated diabetes, Charlson index, previous antidiabetic drugs, should be evaluated.

While there may be an appeal in conducting subgroup analyses, we had decided, a priori, to limit the number of subgroup analyses to avoid issues related to multiple testing as our manuscript already includes several analyses. We had also anticipated that many of such subgroup analyses would be underpowered, resulting in inconclusive findings. For this reason, we have not included such analyses as part of our revised manuscript.

4. Moreover, the difference between the groups in the "duration of diabetes treated" and previous use in metformin should be further elaborated, since for metformin has

been postulated a protective role for cancer and diabetes itself is a risk factor for cancer.

The Reviewer correctly notes that duration of treated diabetes and metformin use may be important potential confounders. It is precisely for this reason that all of our models were adjusted for duration of treated diabetes. As for the purported anti-tumour effects of metformin, this is debated as many of the studies reporting decreased risks had important time-related biases that exaggerated the potential benefits (Suissa & Azoulay. Diabetes Care. 2012 Dec;35(12):2665-73). Nonetheless, we conducted a sensitivity analysis where we included metformin along with other antidiabetic drugs in the model; the adjustment for the use of other antidiabetic drugs produced consistent findings as those observed in our primary analysis (see Figure 2).

5. Table 2: please provide the full adj HR including also the previous use of antidiabetics in the model.

Table 2 includes the fully adjusted hazard ratio for all exposure categories. This analysis did not the use of other antidiabetic drugs. The use of such drugs was not included in the model of our primary analysis due to concerns of collinearity with our exposure variable. However, as described above, the use of other antidiabetic drugs was considered in a sensitivity analysis (see Figure 2).

6. Finally, given the etiology of cancer is complex please try to extend the latency period from 1 to 2 years in a sensitivity analysis.

The extension of the latency period to 2 years is reported with our sensitivity analyses in Figure 2. We note that the HR for pioglitazone was higher in this sensitivity analysis than when using the 1 year latency period (HR: 1.73, 95% CI: 1.26 to 2.39).

Reviewer 5 (Aidan Noon)
Comments

Thank you for submitting this paper to the BMJ. This is an interesting topic and a well written paper.

We thank the Reviewer for the positive comment.

I have three areas of concern.

1. There have been a number of studies looking at the association of pioglitazone and bladder Cancer. The paper does not really explain why this paper is important and worthy of publication in the BMJ. Is pioglitazone still being used in the UK - what were the regulatory actions? Do we want to use this drug more because it is a more useful drug? Should urologists be screening patients that have been exposed to the drug? Is this still a relevant story???

We thank the Reviewer for these comments. Pioglitazone is recommended by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) to be used as a second- or third-line agent in the treatment of type 2 diabetes when metformin is insufficient or contraindicated (Inzucchi et al. Diabetes Care 2015). Pioglitazone has been shown to be beneficial in the treatment of type 2 diabetes given its ability to decrease glycaemia with low risk of hypoglycaemia. Furthermore, it can be used in the treatment of type 2 diabetic patients with renal insufficiency. Therefore, it is still being used in certain patients. Although the use of pioglitazone is currently very limited due to concern about the risk of bladder cancer and, to a lesser extent, heart failure, several important studies have been published in 2015 in which a statistically significant association between pioglitazone and bladder cancer was not observed (Lewis, et al. JAMA 2015;314(3):265-77; Levin D, et al. Diabetologia 2015;58(3):493-504.). These studies received great attention by the media. The consequence of this is that some authors have begun to advocate for the increased use of pioglitazone in clinical practice (Ryder RE. Diabet Med 2015;32(4):e9-e15). However, we believe that the results of these recent studies are affected by important methodological shortcomings and that the increased use of pioglitazone could potential harm patients. Given this context, we believe that our findings will be of great interest to the concerned stakeholders.

2. Given the number of studies and their conflicting studies is this paper definitive in its findings or is it adding to the noise? The statistical analyses appear to be very credible and probably represent the best that can be achieved retrospectively. Why were patients younger than 40 excluded from the study? Patients younger than 40 still get UCC albeit rarely?

We believe that our study addresses the most important methodological shortcomings of the previous studies. Indeed, we have conducted a total of 9 sensitivity analyses, all of which have produced consistent results, further strengthening the conclusions that can be drawn from our study. Moreover, the absence of an association with rosiglitazone suggests that this is likely to be a drug-specific effect. To our knowledge, few observational studies have simultaneously assessed the risk of pioglitazone and rosiglitazone.

We restricted the cohort to patients at least 40 years of age because type 2 diabetes is rarely diagnosed in patients under the age of 40 (it is typically diagnosed in the early 60s, with younger patients diagnosed with diabetes often having type 1 diabetes). This goal was to assemble a homogeneous cohort of patients treated for type 2 diabetes. This approach is consistent with that used in many studies published in this field.

3. Currently there is very little biological data to support the findings cited by the authors. Have there been any animal studies using the drug? What about genotyping the tumours or patients (like aristolochic acid)?

We agree with the Reviewer that the biological evidence supporting the association is controversial, and the debate around this association is still ongoing. In the interest of space, we have provided a succinct summary of the possible biological mechanism in the manuscript (see Page 18, first paragraph).

We agree that genetic studies to determine the types of mutations present in bladder cancer cells among pioglitazone-treated patients would be of interest to determine the types of somatic mutations present and to determine if these mutations differ from those of untreated patients. However, this type of information is unavailable in the CPRD and would require a separate study to assess this question. Certainly, additional studies assessing the pathology observed in patients diagnosed with bladder cancer after being exposed to pioglitazone would provide information on the mechanism by which this drug would induce bladder cancer in humans.

Suggestions for improvement:

4. Please can the manuscript explain what happened to pioglitazone after the controversy of the risk of UCC broke? Was the product withdrawn from the UK or other parts of the world? Is it still being prescribed in the UK? More detail here would improve the appeal of the paper to the journal's general readership.

The pioglitazone story is interesting. Briefly, in June 2011, the Food and Drug Administration published a "Drug Safety Communication" to warn users of a possible 40% increased risk of bladder cancer associated with the use of pioglitazone. This warning followed several clinical and observational studies that have associated the use of pioglitazone with bladder cancer. At that time, the French Medicines Agency suspended the use of pioglitazone. This action was later followed by Germany, but only for new users (for more information, please see: http://www.drugwatch.com/actos/bladder-cancer.php). In the United Kingdom, pioglitazone remained available and continues to be prescribed as monotherapy or as a second- and third-line agent for the treatment of type 2 diabetes.

As of our manuscript now has over 3600 words, we decided to restrict our introduction to a brief history of the pioglitazone controversy. Although a more detailed history of the controversy would be interesting, we ultimately believe that it would be best suited for inclusion as part of a commentary or editorial rather as part of this original research article.

5. How beneficial is pioglitazone to diabetics? For example is this drug very beneficial and therefore it is important to establish its safety so it can be used. If there are other drugs that are equally beneficial - why still study the possible carcinogenic effect of this drug?

As described above, the use of pioglitazone induces a decrease in glycaemia with relatively low risk of hypoglycaemia. Furthermore, pioglitazone can be used in patients with renal insufficiency. Among the current oral hypoglycaemic agents available, only dipeptidyl peptidase 4 inhibitors (i.e., linagliptin) and one of the meglitinides (i.e. repaglinide) can be used in patients with decreased renal clearance. Hence, pioglitazone may be a favourable option that could be used to attain glycaemic control for type 2 diabetic patients with renal insufficiency and recurrent hypoglycaemia. Given these potential benefits, it is important to explore possible adverse effects associated with its use to ensure that stakeholders have a complete understanding of the benefits and risks of this drug. The relevance of safety studies on this drug has been already addressed in our reply to Reviewer 5, Comment 1.

6. Possible biological explanation for the observed effect of pioglitazone: It is difficult to imagine that Urothelial cell carcinoma is being induced after such a short exposure time. Can this be supported by citing other carcinogens in UCC or other cancers? UCC is known after exposure to other drugs e.g. aristolochic acid et al. What are their exposure times???

We thank the Reviewer for his comment. We agree that further studies are needed to understand the mechanism by which pioglitazone may induce bladder cancer, and to explain the increased risk observed relatively soon after treatment initiation. As the Reviewer stated, other carcinogens, such as aristolochic acid are known to increase the risk bladder cancer, but the exposure time required to observe this effect has been poorly described in the literature. Aristolochic acid has been shown to cause DNA mutations in urothelial cells in animal and human studies. However, the mechanism in which pioglitazone may induce bladder cancer is likely different and the exposure time needed to observe this effect may also be different from what has been observed with carcinogens. Due to these uncertainties, we chose not to speculate on the biological mechanisms.

Reviewer 6 (Adam Dunn)

The authors report the results of a population-based cohort study with the aim of identifying an association between the use of pioglitazone and bladder cancer. They find an association that exists for pioglitazone but not for rosiglitazone, demonstrating that the association is specific to pioglitazone and not consistent across the drug class.

This study is designed to address some of the shortcomings of existing studies in the area – in particular a set of methodological issues that may have contributed to different results in previous studies.

I was particularly interested in two aspects of the study, where I thought there may be some issues in the methods. The first was the presence of a sensitivity analysis, which is necessary where there are a series of potentially arbitrary choices about how to decide on the inclusion and exclusion criteria, and the definitions for exposure and outcome. I could not find any specific fault in the sensitivity analysis, and I note that the results were relatively consistent when those decisions were relaxed or modified.

The second aspect was unmeasured confounders, which could invalidate the results. The method used to address unmeasured confounders appears reasonable and I did not find any faults or issues.

1. It may be asking too much to include further analysis in the text, and it does not appear to present a particular risk to the study, but I did note that rosiglitazone use in the cohort dropped off rapidly by about 2007/8 while pioglitazone faced a steadier decline. Is there any chance that further differences in practice between 2007 and 2010 might have contributed to the differences in the outcome measure? Was there a specific reason not to use pairwise comparison?

We thank the Reviewer for this comment. The rapid decline in rosiglitazone prescribing after 2007 was expected, because of concerns regarding cardiovascular risks (mainly because of the meta-analysis by Nissen and Wolski, N Engl J Med. 2007 Jun 14;356(24):2457-71). However, we considered this rapid drop not related to the outcome, since rosiglitazone was not previously associated with bladder cancer. Finally, while the use of rosiglitazone dropped off rapidly after 2007, it was still possible to assess the risk of bladder cancer in patients who were previously exposed to that drug (i.e., the follow-up continued for as long as patients were in the cohort). Thus, it was possible to capture events long after treatment discontinuation, which is important in the context of cancer incidence where the risk may persist long after the drug has been stopped. Finally, it is important to note that all of our models adjusted for calendar year of cohort entry.

Finally, we would also like to note that we did a pairwise comparison between pioglitazone and rosiglitazone. In this direct comparison, we used high-dimensional propensity scores, which included year of cohort entry (Table 3).

2. The comparison with other studies is clear and identifying the biases and methodological differences across the set of existing studies appears reasonable. While I don't think it is necessary to report, there appears to be a reasonably clear association between the conclusions made in the studies reporting on bladder cancer and pioglitazone (refs 4 to 14) and the sources of funding and competing interests in those studies. Beyond the biases that have been reported, it is possible that conflicts of interest are an underlying source of bias that predisposed authors to (a) particular study designs; and (b) conclusion statements that include obvious "spin".

Our discussion focuses on the methodological quality of the previous studies.

As hard as I looked, I was unable to find anything specifically problematic with the manuscript, and I felt that any places where problems may have been introduced were more than adequately covered and described by the authors. I wonder if the BMJ policy on access to patient data is relevant here. Overall, I felt the study was excellent, addressed an important topic, and I could find no faults in the work.

We thank the Reviewer for his positive comments.

Reviewer 7 (Katy Bell)

General Comments

This well written and interesting paper reports on the apparent association between pioglitazone use and incident bladder cancer in a large population based cohort of adults resident in the U.K. The authors could address the following comments to make the paper more convincing.

We thank the Reviewer for her positive comments.

Major Comments

1. From the results presented for year of cohort entry, it appears that the length of follow-up of patients may differ between the categories of TZD users. It is possible that pioglitazone users may have been followed up for longer than non-pioglitazone users, and that this might contribute to a higher rate of incident bladder cancer diagnosis. The multivariate models currently adjust for year of cohort entry, but that may not entirely capture differences in length of follow-up. It would be helpful to have information on the duration of follow-up available for pioglitazone users, rosiglitazone users and non TZD users (added to Table 1 and Supplementary Table 5). Are you also able to adjust for potential differences in length of follow-up in the models?

The Reviewer raises an important point, which perhaps was not clearly articulated in the original manuscript. We agree that differential follow-up between the exposure groups would have introduced bias, as our group has previously described (Suissa & Azoulay. Diabetes Care. 2012 Dec;35(12):2665-73). However, our analytic strategy was designed to avoid this bias. The underlying time axis in all of our Cox proportional hazards models was duration of follow-up. As such, all patients had exactly the same duration of follow-up at each event date (i.e., risk set). This ensured that all exposure groups had the same opportunity of being diagnosed with the outcome while accounting for censoring.

We thank the Reviewer for raising this point, and we have now revised the manuscript to indicate that the underlying time axis for all analyses was duration of follow-up (Page 8, second paragraph).

2. I am also uncertain whether the dose response relationships found with the cumulative duration and cumulative dose variables might be confounded by duration of follow-up – are patients with a lower cumulative duration or dose of pioglitazone also followed up for a shorter period of time, with less chance to develop bladder cancer? Please explain how you have allowed for this in your analysis.

As explained above, since the underlying time axis for all analyses was duration of follow-up, the associations observed for cumulative duration and cumulative dose accounted for this important variable.

3. I think that the pioglitazone versus rosiglitazone head to head comparison theoretically would provide the most compelling evidence for a causal association between pioglitazone and bladder cancer, as there should be a lower risk of imbalance in potential confounders between these two groups of patients. For this comparison I note that the statistical evidence for a difference in association is much less strong (lower confidence limit 1.01 for main model and 0.94 for sub-cohort model). I note from Supplementary Figure 4 that more pioglitazone new users were trimmed due to non-overlapping propensity score distributions than rosiglitazone new users. Did you also do analysis without including propensity scores? Were the sensitivity analyses also done for the head to head comparison?

We agree with the Reviewer that the findings of the head-to-head comparison are compelling. As shown in Supplementary Table 5, the characteristics of pioglitazone and rosiglitazone

users were remarkably similar. Thus, confounding is unlikely to explain the association observed in this analysis.

The use of the high-dimensional propensity score necessitated trimming patients in non-overlapping areas of the propensity score distributions. This is a recommended procedure prior to adjusting for or matching on propensity score (Schneeweiss et al Epidemiology 2009;20(4):512-22). These were patients for whom the probability of being exposed to pioglitazone versus rosiglitazone was either close to 0% or 100%. From a clinical standpoint, these are patients for whom there is no clinical equipoise. We had decided, a priori, not to perform a conventional adjustment (i.e., adjusting for the pre-defined covariates in our outcome model) because we felt that such a method would be suboptimal to the high-dimensional propensity score method (which included all the pre-defined covariates along with another 500 empirically-defined covariates), both in terms of residual confounding and model fit (due to the number of covariates per event with the traditional approach). However, traditional adjustment for the pre-defined covariates yielded similar results (HR: 1.41, 95% CI: 0.96 to 2.07). Finally, we did not perform sensitivity analyses on the head-to-head comparison because this was considered a secondary analysis, and we tried to avoid issues related to multiple testing.

4. Did you consider sensitivity analyses examining associations with a different cancer (or other outcome) you would not expect to be associated with pioglitazone use? If such an analysis demonstrated no association this would add support to your findings that there is a real association with incident bladder cancer.

The Reviewer's suggestion is very interesting. Certainly, the use of a "negative control outcome" can provide additional evidence of possible causal association. However, with respect to cancer, previous studies have associated the use of TZDs with a wide range of malignancies. For instance, in the large PROactive trial, pioglitazone was associated with a lower number of breast cancer events compared to placebo. In contrast, the recent study published in JAMA (Lewis et al. 2015) associated pioglitazone with an increased risk of pancreatic and prostate cancer. Thus, while it may be appealing to assess the effect of pioglitazone and rosiglitazone on other cancers, these drugs may not necessarily have neutral effects.

An alternative approach to a "negative control outcome" is to use a "negative control exposure". For this study, the natural "negative control exposure" was rosiglitazone, a TZD that was not previously associated with an increased risk of bladder cancer in randomized controlled trials. It is precisely for this reason that we replicated all the pioglitazone analyses on rosiglitazone. Reassuringly, pioglitazone was associated with an increased risk of bladder cancer in all analyses, whereas null associations were observed with rosiglitazone.

Minor Comments

5. Please comment on whether all assumptions for the models were tested and whether they were found to hold.

All model assumptions have been adequately tested and appeared to hold. This is now reported on Page 9 (first sentence on the page) of the revised manuscript.

6. Why were the data for year of cohort entry suppressed for new pioglitazone users for 2000 and 2001? Why were there no patients started on rosiglitazone after 2010?

As per the policies of the Independent Scientific Advisory Committee of the Clinical Practice Research Datalink, all cells with a count smaller than or equal to 5 must be suppressed to protect the privacy of patients. This is specified in the footnotes of the tables.

Rosiglitazone was withdrawn from European market in July 2010 due to cardiovascular safety issues. Therefore, there were no patients receiving this drug following that year.

7. Please make it clearer that pioglitazone and rosiglitazone users in Table 1 and Supplementary Table 1 respectively refer to new users at cohort entry, whereas Table 3, and Supplementary Table 5 refer to new users at any time from cohort entry until end of follow-up.

We thank the Reviewer for this point. We have now updated the titles of Table 1, Supplementary Table 1, and Supplementary Table 5 to clarify that TZD exposure was measured at cohort entry.

8. In the Methods, Based cohort, please provide justification for why you excluded patients with more advanced form of type 2 DM (as indicated by prescription of insulin). You mention this in the Discussion in relation to other studies' short comings, but it would also be helpful to explain this here.

The use of insulin as a first-line treatment is atypical, and would strongly suggest that such patients have advanced disease. It is possible that these patients were prevalent users, having received antidiabetic drugs prior to their registration with the CPRD. These patients accounted for 1.6% of our initial cohort. The inclusion of such patients in the cohort would result in several important biases, including prevalent user bias (due to the inclusion of prevalent users and the corresponding depletion of susceptibles) and, to a lesser extent, confounding by disease severity. The inclusion of prevalent users represents a crucial shortcoming of previous studies in this area. For this reason, we restricted inclusion to initiators of non-insulin antidiabetic drugs.

9. Please define immortal time bias

Immortal time bias occurs when misclassifying the unexposed person-time as exposed person-time. This time is also immortal, because for a patient to be considered exposed, he or she had to survive to be able to receive a prescription. Thus, the inclusion of this misclassified immortal person-time in the denominator of the rate of the exposed group can lead to an underestimation of the rate ratio. This is now briefly explained in the manuscript (Page 16, last paragraph).

10. For the dose-response relationship for pioglitazone, was the risk of bladder cancer with cumulative doses below 10,500 mg, as well as above 28,000 mg higher than for doses in between? If so, do you have an explanation for this?

This is indeed an interesting finding. The association with the lower cumulative doses may reflect a treatment strategy where lower doses are given when pioglitazone is prescribed in dual or triple therapy with other antidiabetic drugs among patients with more advanced disease.

11. Figures need titles as well as legends.

Figure captions are reported on Page 27 of the manuscript. For the Supplemental Figures, the titles and legends are included in the Supplemental file alongside the figures.

Reviewer 8 (Frida Emanuelsson)

Dear authors and editors
Thank you for the opportunity to review this manuscript. In general I think this is a very well written and still relevant issue to address. As the authors mention there has been published contradiction reports on the subject and additional, well-designed studies are needed. This is an observational registry study, based on a large population which yields a number of cases of the rare adverse event but is also limited by its design of a registry study.

We thank the Reviewer for her positive feedback.

1. The authors mention they have controlled for a number of confounders such as smoking and HbA1c. However, this Hba1c was one value, measured before inclusion. I can't see how one arbitrary HbA1c value could say much about the general control over years of the individual patient. Also, if I understand the design correctly, patients were included in the cohort if they were to switch between therapies / initiating new drug class - then I would expect all patients to be not in control/ high in Hba1c at that point of time?

We thank the Reviewer for this comment. Indeed, measuring HbA1c at a single point in time may lead to some residual confounding. As described above, we conducted an additional sensitivity analysis using a marginal structural model, a method that allowed us to adjust for the potential time-dependent confounding of HbA1c and other covariates during follow-up. This sensitivity analysis yielded findings that were consistent with those of the primary analysis.

A detailed description of the marginal structural model method is available in the Appendix I in the Supplemental file and the results for pioglitazone and rosiglitazone are presented in Figure 2.

2. I suggest occupational history also to be a relevant confounder for BC that is not commented on tin the text.

Occupational exposure is an important risk factor for bladder cancer. Unfortunately, the CPRD database does not provide sufficient information about occupational history that allows us to control for this confounder. This is acknowledged as one of the study limitations (see Page 15, last pargraph and Page 16, top of the page). However, while this variable is associated with the outcome, it is not clear whether it is associated with the exposure, and therefore the absence of this variable is unlikely to explain the observed association.

3. The authors mention that the pioglitazone users were more likely to have prior bladder conditions a baseline - could you maybe comment on this, what kind of conditions, what do you think is the reason and if it may affect the results?

As also noted by Reviewer 8, TZD users were more likely to have bladder conditions at baseline. These were defined by a history of infections (i.e. cystitis) and bladder stones. To assess the impact of these conditions on our findings, we performed a sensitivity analysis where we excluded patients with bladder conditions at any time prior to cohort entry and censored patients at the time of a new diagnosis for these conditions during follow-up. The results of this sensitivity analysis generated findings that were consistent with those of our primary analyses (Figure 2).

4. In the beginning of the discussion it is stated that there was up to 15 yrs of follow-up - I don't understand how, if follow up started the year after cohort entry (i.e. 2001) and until 31 July 2014 as is stated in the methods section?

We thank the Reviewer for this comment and understand the confusion. While the earliest calendar year for follow-up (i.e., person-time at risk) was 2001 (because of the 1 year lag period), exposure to pioglitazone and rosiglitazone was possible as early as January 1, 2000 (though patients would have been considered exposed starting January 1, 2001). Thus, the study period was from January 1, 2000 to July 31, 2014, a total of 174 months or 14.5 years. We revised the manuscript to reflect this more accurate number (Pages 9 and 15).

5. In the results section you mention the incidence rates for BC for pioglitazone users vs other British T2 diabetics. For the readers reference I think it could be nice to shortly mention in the discussion if the incidence matches for other T2DM populations and the general population, to put it into perspective.

The observed bladder cancer rate was not only consistent with that observed in other British patients with type 2 diabetes but also with that observed in Americans with type 2 diabetes. A recent US cancer registry study estimated a rate of 114/100,000 (95% CI: 99.4-130.4) per year among patients with type 2 diabetes (Int. J. Cancer. 2013: 132, 2186–2191.); the 95% CI of this estimate overlaps with that reported in the present study. While rates are available for the general population, we have not cited such studies as type 2 diabetes is a risk factor for bladder cancer and we therefore anticipate rates in our study to be higher than those observed in the general population.