



Pharmacoepidemiological study protocol Pioglitazone-5020/ER-9531

Pioglitazone Use and Risk of Bladder Cancer: a Systematic Review and Meta-Analysis of Observational Studies

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Protocol number: Pioglitazone-5020/ER-9531

Sponsor: Takeda Development Centre Europe Ltd.

Protocol version: v1.2

Protocol date: 27 October 2016

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1 List of abbreviations

List of main abbreviations used in the study protocol

ATC code	Anatomical therapeutic chemical classification system code
CI	Confidence interval
HR	Hazard ratio
KPNC	Kaiser Permanente Northern California
OR	Odds ratio
EU PAS Register	European Union electronic Register of Post-Authorisation Studies
PPAR γ	Peroxisome proliferator activated receptor- γ
RR	Relative risk
T2DM	Type 2 Diabetes Mellitus
TZD	Thiazolidinediones
UK	The United Kingdom
US	The United States

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Financial support

This study is funded by Takeda Development Centre Europe Ltd.

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2 Abstract

Title. Pioglitazone Use and Risk of Bladder Cancer: a Systematic Review and Meta-Analysis of Observational Studies.

Rationale and background. Several studies have been published investigating the possible risk of incident bladder cancer in type 2 diabetes mellitus patients treated with pioglitazone with conflicting results. Furthermore, meta-analyses of published studies have been conducted, suggesting that pioglitazone use is associated with a modest but significant increase in the risk of bladder cancer. Most previous meta-analyses were conducted using studies published prior to 2013. Considering that more long-term observational studies investigating the possible risk of pioglitazone in humans have been published since 2013, there is a need to review the accumulated real world evidence and update the previous meta-analyses.

Research question and objectives. The primary research question is whether type 2 diabetes mellitus patients treated with pioglitazone are at a higher risk of bladder cancer compared to type 2 diabetes mellitus patients who are not treated with pioglitazone. The secondary research question is whether the risk of bladder cancer is increased by cumulative exposure duration or cumulative dose of pioglitazone.

Study design. For studies to be eligible in this meta-analysis, they have to:

- Be an observational study: cohort study, case-control study, nested case-control study (open label extension of clinical trials are not eligible).
- Include analysis comparing pioglitazone users vs. non-users (Reference group defined as never use of pioglitazone including use of any other anti-diabetic medications) with respect to bladder cancer outcome.
- Involve human subjects with type 2 diabetes mellitus.
- Full text needs to be available, i.e., the article needs to have been published fully in a peer-reviewed journal.
- The study must present a measure of association between bladder cancer and pioglitazone use (odds ratio, relative risk or hazard ratio), or sufficient data to enable estimation of hazard ratio
- Abstracts should be available in English.

Variables. The following data will be extracted from the included studies:

- Study type (e.g. cohort, case-control, nested case-control)
- Authorship and year of publication
- Country or region of source of data
- Study setting (database used)
- Study period
- Follow-up in years
- Patient mean age
- Percentage of male patients
- Study cohort size
- Outcome identification
- Number of bladder cancer cases
- Exposure information: cumulative dose and duration of pioglitazone and subgroup analysis results
- Adjusting covariates used when estimating the association of risk between pioglitazone exposure and bladder cancer.

Assessment of the risk of bias in individual studies. The validity of all included source studies will be evaluated and the risk of bias in each study will be assessed. This process will involve the following considerations:

- Control for confounding by indication/channelling bias
 - Were statistical methods used to adjust for differences in patient baseline characteristics between pioglitazone exposed and unexposed groups?
- Immortal time bias
 - Does follow-up time include immortal time and can this introduce bias?
- Information bias
 - Was the drug exposure clearly defined in the paper? Does this differ between comparison groups?
 - Was the outcome identification clearly defined in the paper? Does this differ between groups?
 - Was censoring of follow-up described? Does this differ between comparison groups?
- Was the effect of possible bladder cancer latency tested by excluding early cancers (e.g. first 6 or 12 months)?
- Was the robustness of study results tested with other sensitivity analysis?

Data synthesis and meta-analyses. Hazard ratio will be the common measure of association that will be extracted from each study, or derived based on available data. Combined estimates will be derived using primarily a random-effects model and repeated secondarily using a fixed-effects model (sensitivity analysis).

Communication of study results. The principal investigator together with the co-investigators will write a study report. This report will be delivered to the sponsor (Takeda). The results will be communicated in appropriate scientific venues as abstract and poster. The final results will be published in a peer-reviewed journal.

3 Amendments and updates

In the event of protocol amendments, the date, protocol section, description of update and the reason for the update will be documented in the table below.

Number	Date	Section of study protocol	Amendment or update	Reason
1.	Date	Text	Text	Text
2.	Date	Text	Text	Text

4 Milestones

Milestone	Planned date
Protocol development	27 October 2016
Literature review	31 October 2016
Statistical analysis plan development	31 October 2016
Data-analyses	30 November 2016
Study report	31 December 2016
Publication 1 st draft	31 January 2017
Publication 2 nd draft	28 February 2017

5 Rationale and background

The burden of diabetes has been rising with the increase in the prevalence of diabetes. Currently, type 2 diabetes presents a global public health problem not only in industrialized countries but across the globe. In 2014, an estimated 422 million individuals or 8.5% of the world's population had diabetes in 2014¹. It is estimated that 29.1 million people had diabetes in the United States (US) in 2014 including 21 million diagnosed and 8.1 million undiagnosed cases². In the US alone, direct medical costs of \$176 billion and indirect costs of \$69 billion were spent on diabetic patients care in 2012³.

Pioglitazone is an agonist of peroxisome proliferator activated receptor- γ (PPAR γ) in the class of thiazolidinedione (TZD) to treat type 2 diabetes mellitus (T2DM). Effective glycaemic control has been observed for pioglitazone in both diabetic and pre-diabetic population. The Actos Now for Prevention of Diabetes (ACT NOW) clinical trial showed that pioglitazone reduced the risk of T2DM conversion in adults with impaired glucose tolerance by 72%^{4,5}. Pioglitazone has been shown to be efficacious in glycaemic control in type 2 diabetes patients alone or in combination with metformin, sulfonylurea or insulin⁶⁻⁹.

The use of pioglitazone raised a safety concern after an increase in the risk of bladder cancer was observed in exposed male rats¹⁰. Similar increase was not observed in female rats or mice of both genders¹⁰. Several studies have been published investigating the possible risk of pioglitazone in humans with conflicting results¹¹⁻¹⁷. Furthermore, several meta-analyses of published studies have been conducted, suggesting that pioglitazone use is associated with a modest but significant increase in the risk of bladder cancer¹⁸⁻²³. All

previously mentioned meta-analyses were conducted using studies published prior to 2013. More observational studies have been published since then and there is a need to review the accumulated evidence²⁴⁻²⁶.

Takeda, the sponsor of this study, has conducted and completed additional large observational studies to further investigate the possible association between pioglitazone and bladder cancer. A long term 10-year follow up study using the Kaiser Permanente Northern California (KPNC) in the United States (US) following a diabetic cohort of 193 099 persons from 1997-2002 until December 2012 was completed and published in 2015²⁴. This US based KPNC study found no association between pioglitazone and bladder cancer (adjusted hazard ratio (HR), 1.06; 95% confidence interval (CI), 0.89-1.26). In addition, the KPNC study also carried out a nested case-control study of 928 persons for complete bladder cancer related medical history, and no association was observed in this nested case-control study either (adjusted odds ratio (OR), 1.18; 95% CI, 0.78-1.80). Another European cohort study pooled six large European databases (Pan EU study) and selected 56,337 eligible pioglitazone users matched to 56,337 non-users for analysis. No association was also observed in this study (adjusted OR, 0.99; 95% CI, 0.75-1.30). Meanwhile, there are other observational studies that have been published since July 2013. Recent Tuccori et al. 2016²⁶ meta analysis publication found that pioglitazone was associated with increased bladder cancer risk as compared to other antidiabetic drugs among 145 806 diabetic patients in the United Kingdom (UK). Conversely, Levin et al. 2014²⁴ did not find a statistically significant association between pioglitazone and bladder cancer.

New evidence has emerged since the publication of the Turner et al. 2013 paper that have not been incorporated in the latest observational knowledge by meta-analysis methods. This new body of evidence is very important now as previous meta-analyses were limited by the small number of studies included. For instance, in the Turner et al. 2013 review, only eight observational studies were included for pioglitazone, among which one long term observational US based study (KPNC Bladder study) had its follow-up data later updated and the final results reversed (from significant risk to no risk). Also, meta-analysis is subject to publication bias as articles of non-significant results tend to be published years after the ones with significant results. Turner et al. 2013 did not evaluate publication bias due to small sample size. In addition, Turner et al. 2013 used fixed-effects model for analysis based on small I^2 statistic for heterogeneity, which is heavily biased by small sample sizes²⁷. The choice of model might have an impact on the magnitude and direction of observed risk. Furthermore, 3 of the 8 observational studies included were from Taiwan. As a result, patients in the Taiwanese population could have more contribution than patients in other populations. The other referred meta-analyses review the association of bladder cancer and pioglitazone with slightly different methods as compared to Turner et al. 2013 They use the random-effects and address the publication bias. However, all these meta-analyses employ largely the same relatively small number of studies, underlining the need to update knowledge with observational data from newer studies. Overall, there is a need to update the observational evidence generated by previous meta-analysis publications using a more appropriate model of choice so we can provide the best possible observational evidence on pioglitazone use and risk of bladder cancer. Moreover, this new meta-analysis will generate additional data that can be used in the evaluation of risk benefit profile of pioglitazone.

6 Research questions and objectives

The objective of this meta-analysis is to assess the association between bladder cancer and exposure to pioglitazone among subjects with T2DM in a meta-analysis of observational studies. To this end, this meta-analysis will answer the following questions:

Primary research question: Are T2DM patients treated with pioglitazone at higher risk of bladder cancer compared to T2DM patients who are not treated with pioglitazone?

Secondary research question: Is the risk of bladder cancer increased by increasing cumulative exposure duration or increasing cumulative dose of pioglitazone?

7 Research methods

7.1 Study Design

This meta-analysis will be based on a systematic and comprehensive literature review that will be conducted to identify eligible observational studies from peer-reviewed scientific journals. Studies published before September 30, 2016 will be identified using the search strategy detailed below. Studies included in the meta-analysis resulting from the electronic search will be included in the selection process and screened for eligibility.

7.2 Search strategy

The MEDLINE electronic database will be searched with the following terms:

- (Pioglitazone OR Actos OR Thiazolidinedione) AND ("Bladder cancer" OR "urinary" OR "bladder carcinoma in situ" OR "Urinary Bladder Neoplasms"[Mesh])

For studies identified in the search, they have to meet the following criteria to be eligible:

- Be an observational study: cohort study, case-control study, nested case-control study (open label extension of clinical trials are not eligible).
- Include analysis comparing pioglitazone users vs. non-users (Reference group defined as never use of pioglitazone including use of any other anti-diabetic medications) with respect to bladder cancer outcome.
- Involve human subjects with T2DM.
- Full text needs to be available, i.e., the article needs to have been published fully in a peer-reviewed journal.
- The study must present a measure of association between bladder cancer and pioglitazone use (OR, relative risk (RR) or hazard ratio (HR)), or sufficient data to enable estimation of HR
- Abstracts should be available in English.

Previous meta-analysis resulting from the electronic search will be used to identify additional eligible records to be included in this review. In addition, the reference section of each included observational study will be checked to help ensure complete ascertainment of studies.

7.3 Selection process

We will conduct a systematic literature review in MEDLINE electronic databases to identify published observational studies from peer-reviewed journals. The search strategy described in section 7.2 will be used for qualifying studies until September 30, 2016. Titles and abstracts yielded by the search will be screened against the inclusion criteria by two review authors independently. Qualifying or uncertain studies from title and abstract review will undergo further full text review and decision will be made on whether or not these meet the inclusion criteria. Any disagreement between the review authors will be solved through discussion or by appeal to a third review author. For studies to be excluded, reasons for exclusion will be recorded as well. Neither of the review authors will be blinded to the journal titles or to the study authors or institutions.

In the case that several publications are based on substantially overlapping data, only the most comprehensive one will be taken into account in the meta-analysis, as applicable. If several publications are based on the same study with data updated over years, only the most recent publication will be included in this meta-analysis.

7.4 Extracted variables

The following demographics data will be extracted from the included studies:

- Study type (e.g. cohort, case-control, nested case-control)
- Authorship and year of publication
- Country or region of source of data
- Study setting (database used)
- Study period
- Follow-up in years
- Patient mean age
- Percentage of male patients
- Study cohort size
- Outcome identification
- Number of bladder cancer cases
- Exposure information: cumulative dose and duration of pioglitazone and subgroup analysis results
- Adjusting covariates used when estimating the association of risk between pioglitazone exposure and bladder cancer.

The primary outcome variable is incident bladder cancer. Crude and adjusted bladder cancer outcomes will be extracted from each study for pioglitazone users vs. non-users. Cumulative dose and cumulative duration of pioglitazone exposure will be extracted for detailed information regarding exposure as well if applicable.

7.5 Assessment of risk of bias in individual studies

The validity of all included source studies will be evaluated and the risk of bias in each study will be assessed. This process will involve at least the following considerations:

- Control for confounding by indication/channelling bias
 - Were statistical methods used to adjust for differences in patient baseline characteristics between pioglitazone exposed and unexposed groups?
- Immortal time bias
 - Does follow-up time include immortal time and can this introduce bias?
- Information bias
 - Was the drug exposure clearly defined in the paper? Does this differ between comparison groups?
 - Was the outcome identification clearly defined in the paper? Does this differ between groups?
 - Was censoring of follow-up described? Does this differ between comparison groups?

- Was the effect of possible bladder cancer latency tested by excluding early cancers (e.g. first 6 or 12 months)?
- Was the robustness of study results tested with other sensitivity analysis?

A judgement as to the possible risk of bias will be made for each of the pre-specified questions based on the extracted information, rated as 'high risk' or 'low risk'. If there is insufficient detail reported in the study we will judge the risk of bias as 'unclear'. These judgements will be made independently by two review authors. Disagreements will be resolved first by discussion and then by consulting a third author for arbitration. A table of potential biases across studies will be created.

7.6 Data management and synthesis

R language (<http://www.r-project.org>) will be used for data management for creating the analysis database and in statistical analysis for creating tabulations and graphics as well as in all statistical modelling. R language is described in more detail in report "R: Regulatory Compliance and Validation Issues: A Guidance Document for the Use of R in Regulated Clinical Trial Environments" (www.r-project.org/doc/R-FDA.pdf, read 23 May 2016). The raw dataset and statistical programs used for generating the data included in the study report will be kept in electronic format and be available for auditing and inspection.

Missing data

The study must present a measure of association between bladder cancer and pioglitazone use [OR, relative risk (RR) or hazard ratio (HR)], or sufficient data to enable estimation of HR. For studies with missing such measure of association, statistical methods will be used to derive these estimates for the effect size of ever versus never exposure to pioglitazone. Such studies with their derived estimates will be included together in a sensitivity analysis.

7.7 Statistical analyses

A separate statistical analysis plan (SAP) including detailed statistical analysis outputs will be produced before undertaking the analysis.

Presentation of data

The search procedure and inclusion of studies will be presented in a step-by-step diagram indicating the number of identified and eligible studies at each step. The step-by-step diagram will include the number of records in following parts:

- Records identified through each database and additional records identified through other sources
- Records after duplicated have been removed
- Records screened and excluded after screening
- Full-text articles assessed for eligibility and excluded with reasons
- Studies included in qualitative synthesis (full text review)
- Studies included quantitative synthesis (meta-analysis) with reasons for exclusions

All studies included in the qualitative and quantitative synthesis will be presented in a table showing the study type, authorship of the study, year of publication, study setting (database used), country, study period, follow-up in years, patient mean age, percentage of male patients, study cohort size, outcome

identification, number of bladder cancer cases, exposure information: cumulative dose and duration (if available) and adjusting covariates. In addition, the measure of effect sizes in each study will be presented in a forest plot.

Assessment of heterogeneity

Heterogeneity across studies will be assessed by considering the variability in participant factors among studies and among statistical methods used. Statistical heterogeneity will be tested using the Chi² test (significance level: 0.1) and I² statistic (0% to 40%: might not be important; 30% to 60%: may represent moderate heterogeneity; 50% to 90%: may represent substantial heterogeneity; 75% to 100%: considerable heterogeneity). If high levels of heterogeneity among the trials exist (I² >=50% or P<0.1) the study design and characteristics in the included studies will be reviewed and the possible source of heterogeneity discussed.

Combined estimates

Combined meta-analysis HRs will be derived using a random effects model as the primary approach. The results will be accompanied in the forest plot showing results from individual studies.

In the case there are studies that use data from various countries with country-specific and pooled results, the overall pooled estimate will be used in the primary analysis.

Using studies in which the results regarding cumulative duration are available, the following cumulative duration categories of pioglitazone use will be pooled across studies and compared to never use category: <12 months, 12-24 months, >24 months.

Using studies in which the results regarding cumulative dose are available, the following cumulative dose categories of pioglitazone use will be pooled across studies and compared to never use category: <10500 mg, 10500mg - 28000 mg, >28000 mg.

Assessment of publication bias

Publication bias will be studied with the funnel plot and tested using Begg's rank correlation test and Egger's regression test.

Sensitivity analyses

1. Studies prone to high risk of bias after applying the assessment process explained in section 7.5 will be excluded from the meta-analysis.
2. A Bayesian random-effects model will be applied. The Bayesian model will be specified further in the SAP.
3. Individual country specific estimates from multi-country studies will be used instead of the common pooled estimates. The combined meta-analysis HR will be estimated using a Bayesian random-effects model with a higher order hierarchical model. The Bayesian model will be defined further in the SAP.
4. The primary analysis will be repeated using a fixed-effects model (The results will be accompanied in the forest plot showing results from individual studies).
5. For studies not reporting results for never versus ever exposure to pioglitazone, estimation methods will be used to derive estimates for the missing effect size and will be included as a sensitivity analysis into the primary analysis.
6. The primary analysis will be performed separately for case control studies and for cohort studies.

7.8 Quality control

This meta-analysis study will be conducted as specified in the study protocol. The principal investigator and the sponsors of the study must approve all revisions to the protocol. All changes to the protocol shall be properly documented as protocol amendments.

EPID Research will perform a review on the selected publications, including the inclusion of studies and gathered data.

All programs for data management and data analyses will be written by study statistician(s). Quality control check of these programs will be carried out by a statistician other than the one who writes the program. All processes from data management leading to dissemination of study results will undergo quality control checks for programs, result tables and written text. A detailed audit trail of all documents (programs, result tables, reports) along with quality control processes will be maintained.

7.9 Limitations of the research methods

In previous meta-analyses of the association of bladder cancer and the use of pioglitazone, the results have been conflicting. Pooling such conflicting results together leads to an estimate that is a weighted average over estimates in each sub-study. However, the interpretation of the pooled estimate might be difficult, as there might be certain issues that explain the conflicts in the results. For example, if there was some source of bias in certain studies, a pooled estimate would not give means to make stronger conclusions about the association of bladder cancer and the use of pioglitazone.

8 Plans for disseminating and communicating study results

The principal investigator together with the co-investigators from the EPID Research will write a final study report. This report will be delivered to the sponsor (Takeda). The results will be communicated in appropriate scientific venues. Final results will be published in a peer-reviewed journal. Also, the final results may be shared with Regulatory Authorities if deemed appropriate or required.

9 References

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10 Approvals

We have reviewed this study protocol (ER-9531 Version 1.2, dated 27 October 2016) and agree to its terms by signing it.

Role	Name	Address	Date	Signature
Principal investigator	Pasi Korhonen	EPID Research Oy Metsänneidonkuja 12 FI-02130 Espoo Finland		

11 Study registration

The study information, including the study protocol, amendments to the protocol and final study report will be registered in the European Union electronic Register of Post-Authorisation Studies (EU PAS Register).

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