HHS Public Access

Author manuscript

J Urol. Author manuscript; available in PMC 2017 August 01.

Published in final edited form as:

J Urol. 2016 August ; 196(2): 343–348. doi:10.1016/j.juro.2016.01.089.

Association between itraconazole, a hedgehog-inhibitor, and bladder cancer

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Abstract

Purpose—Activation of Hedgehog (Hh) signaling has been implicated in early stages of bladder cancer development, while loss of Hh signaling has been described during progression to more invasive disease. Itraconazole, an antifungal, is the only azole known to be a potent Hh pathway antagonist. We evaluated whether itraconazole use is associated with bladder cancer risk or progression.

Materials and methods—A case-control study nested within a United Kingdom database included 13,440 bladder cancer cases and 52,421 matched controls between 1995–2013. Use of itraconazole and other azoles was measured in number of prescriptions. Conditional logistic regression estimated adjusted odds-ratios (AOR) and 95% confidence intervals (CI) for the association of bladder cancer with ever use and increasing number of prescriptions for itraconazole. Logistic regression was used to determine whether relative to other azoles, if itraconazole use among patients diagnosed with bladder cancer is associated with invasive bladder cancer requiring cystectomy.

Results—Use of itraconazole was not associated with the risk of bladder cancer relative to never use (ever use: AOR 0.89, 95%CI 0.70–1.14; ≥4 prescriptions: AOR 0.87 [0.42–1.81]). However, among patients diagnosed with bladder cancer, there was a significant increased risk of bladder cancer requiring cystectomy with itraconazole use (ever use: AOR 2.05 [1.12–3.38]; ≥2 prescriptions: AOR 2.30 [1.12–4.72]).

Conclusion—Inhibition of the Hh pathway with itraconazole was not associated with risk of bladder cancer overall, but was associated with higher risk of invasive bladder cancer requiring

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Conflicts of interest: R.M reports personal fees from Takeda, outside the submitted work. F.I.S. reports research funding from Takeda, outside the submitted work. J.D.L. reports personal fees from Janssen and Pfizer, outside the submitted work. All remaining authors have declared no conflicts of interest.

cystectomy. These data provide clinical evidence supporting the role of Hh signaling in regulating bladder cancer progression.

Keywords

bladder cancer; hedgehog pathway; itraconazole; pharmacoepidemiology

INTRODUCTION

The Hedgehog (Hh) signaling pathway is a developmental pathway that plays an important role in human embryogenesis [1]. Constitutive activation of this pathway has been implicated in oncogenesis, particularly bladder carcinogenesis [2]. Carcinoma of the urinary bladder arises from the uroepithelium, and is the 9th most common cancer worldwide with a global prevalence of 2.7 million [3]. Recently, Hh-expressing urothelial basal stem cells have been identified as the cell of origin in bladder carcinoma [4]. While Hh expression promotes epithelial proliferation early in bladder cancer development, loss of expression has been associated with progression into invasive disease [5]. Therefore, inhibition of the Hh signaling pathway could have differential effects on bladder cancer risk or progression.

Itraconazole, a currently available oral antifungal agent, is a potent Hh pathway antagonist by a mechanism separate from the inhibition of fungal-mediated synthesis of ergosterol [6]. Itraconazole has also been suggested to inhibit angiogenesis in tumor cells[7]. There are no available data examining the effect of itraconazole exposure on the risk or progression of bladder cancer.

Given that other drugs in the azole class are not inhibitors of the Hh pathway, we examined the risk of bladder cancer in users of itraconazole and other azoles. We also examined whether itraconazole use among patients diagnosed with bladder cancer is associated with more invasive bladder cancer requiring cystectomy.

MATERIALS AND METHODS

Data source

The Health Improvement Network, THIN, is a computerized medical records database representative of the broader United Kingdom (http://www.thin-uk.com/). The database currently contains the electronic records of over 11 million patients. Data available in THIN include demographic information, medical diagnoses, drug prescriptions, lifestyle characteristics such as smoking status, and other measurements recorded by general practitioners (GPs) such as height and weight. Medical diagnoses entered into the database are recorded using Read codes, the standard primary care classification system in the UK [8]. Data quality is monitored through routine analysis of the entered data [9]. The accuracy and completeness of THIN data is well documented [10,11] and the database has been previously used to study the pharmacoepidemiology of bladder cancer [12,13].

Study design and population

We conducted a nested case-control analysis within THIN. The case-control design is computationally efficient and produces odds ratios (ORs) that are unbiased estimates of incidence rate ratios [14]. All patients registered with a THIN general practitioner from 1995 to 2013 were eligible for inclusion. Follow-up started at the later of either the date the THIN practice started using the electronic medical record (Vision software) or the date at which the patient registered with their general practitioner and ended on the index date (described below). The study protocol was approved by the University of Pennsylvania's Institutional Review Board and the United Kingdom's Scientific Review Committee.

Case-control selection

From the source cohort, cases were defined as individuals with at least one diagnostic code for bladder cancer during the follow-up period (See Supplementary Table)[15]. Subjects with a diagnosis of bladder cancer within the first 6 months of the follow-up period were excluded to avoid misclassification of prevalent bladder cancer as incident bladder cancer [11,16]. Selection of controls was based on incidence density sampling [17]. For each individual with bladder cancer, up to four controls were randomly selected after matching on age (±5 years), sex, practice site, duration of follow-up, and calendar period. Additionally, each control subject could not have been diagnosed with bladder cancer as of the date of diagnosis of bladder cancer of the matched case subject. The date that the case subject was first diagnosed with bladder cancer served as the index date for both the case subject and for the matched control.

Exposure definition

Exposure to itraconazole was defined as receipt of at least one oral prescription for itraconazole at least 1 year before the index date. Identical definitions were used to define exposure to other azoles (fluconazole, miconazole, ketoconazole, and voriconazole). We did not consider any azole use in the year immediately prior to the index date to mininimize the possibility that azoles were prescribed due to non-specific symptoms of undiagnosed bladder cancer (reverse causality).

Statistical analysis

Conditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for the association of itraconazole use and risk of bladder cancer, adjusted for confounders [18]. The reference group in this analysis consisted of subjects without documented use of azole anti-fungal medication prior to the index date. In addition to age, sex, practice site, duration of follow-up, and calendar period, on which logistic regression analyses were conditioned, we included 7 factors *a priori* as confounding variables. These 7 variables included smoking, obesity (body mass index ≥30kg/m^2), diabetes mellitus, use of diabetes medications (metformin, insulin, or thiazolidinediones), chronic use of aspirin or non-steroidal anti-inflammatory medications, and recurrent bladder infections, given the association of these variables with the risk or detection of bladder cancer.

We also examined the risk of bladder cancer with increasing exposure to itraconazole. We measured exposure in terms of number itraconazole prescriptions starting with the first

prescription that defined ever use of itraconazole. The reference group for this analysis was never use of itraconazole. Similar analyses were performed to examine the risk of bladder cancer with use of other azole anti-fungal medications.

In an analysis limited to users of any azole, we examined the association of bladder cancer with itraconazole treatment relative to treatment with other azole anti-fungals. Due to the fewer number of cases exposed to an azole in this subset analysis, unconditional logistic regression was used adjusted for age, sex, calendar time, and duration of follow-up, as well as the pre-specified set of confounders.

In a secondary analysis limited to case subjects with bladder cancer, we evaluated the potential effect of itraconazole treatment on the risk of bladder cancer progression requiring cystectomy. In this analysis, bladder cancer cases with subsequent surgical codes for cystectomy were compared to controls without cystectomy.

STATA version 13.0 was used for statistical analyses (StataCorp, CollegeStation, TX). All statistical tests were two-sided.

RESULTS

13,440 case subjects with bladder cancer and 52,421 matched controls were identified. The median duration of follow-up was 6.0 years. The case subjects were more likely than controls to have a history of smoking (57.1% vs. 43.4%), recurrent bladder infection (10.9% vs. 4.6%), and diabetes mellitus (13.7% vs. 10.7%) (Table 1).

Itraconazole was used by 86 (0.6%) cases and 354 (0.7%) controls. The median duration of use was estimated to be 4 weeks (IQR 2.0–4.3) in cases and 4 weeks (IQR 1.1–5.0) in controls. In the adjusted model, use of itraconazole was not associated with the risk of bladder cancer (AOR 0.89, 95% CI 0.70–1.14) (Table 2). In analysis of increasing levels of itraconazole exposure, there was no evidence of a statistically significant decreased risk of bladder cancer with increasing number of itraconazole prescriptions. For example, for patients with ≥4 itraconazole prescriptions, the adjusted OR was 0.87 (95% CI 0.42–1.81) (Table 2). Similarly, there was no association between ever use (AOR 1.06, 95% 0.97–1.16) or increasing use (≥4 prescriptions: AOR 1.15, 95% CI 0.95–1.39) of the other azoles and the risk of bladder (Table 2).

When we compared bladder cancer risk in itraconazole users relative to other azole users (Table 3), there was no evidence of a statistically significant decreased risk of bladder cancer associated with use of itraconazole (AOR 0.84, 95% CI 0.65–1.09) or increasing number of itraconazole prescriptions (≥4 prescriptions: AOR 0.84, 95% CI 0.40–1.75).

Among 13,440 patients with incident bladder cancer, 1,004 underwent subsequent cystectomy. The median time from bladder cancer diagnosis to cystectomy was 128 days (IQR 62 to 328 days). In this secondary analysis limited to patients with bladder cancer (Tables 4 and 5), itraconazole use but not other azole use was associated with a statistically significant increased risk of bladder cancer requiring cystectomy (ever vs. never use of itraconazole: AOR 2.05 [1.12–3.38]; ever vs. never use of other azoles: AOR 0.83 [0.60–

1.15]). Similar results were observed in the comparison of itraconazole to other azole use (AOR 2.30 [1.12–4.72]). These results were also unchanged in post-hoc analyses after adjustment for prior immune-mediated disease (rheumatoid arthritis, inflammatory bowel disease, or connective tissue disease), steroid use, and diagnosis with other solid cancers (before adjustment: HR 2.05 [1.12–3.78], after adjustment: HR 2.10 [1.14–3.87]).

DISCUSSION

Activation of the hedgehog (Hh) pathway occurs in most non-muscle invasive bladder cancers and 50% of muscle-invasive cancers [2]. The negative regulator of Hh signaling, Ptch1, is located on chromosome 9q, loss of which is a common and early event in bladder tumorigenesis [19]. Furthermore, in murine models, Hh-expressing basal cells have recently been identified as probable bladder cancer stem cells [4]. Thus, hedgehog signaling could serve as a potential target for the chemoprevention of bladder cancer. Contrary to these pre-clinical data, in this case-control analysis using a large UK medical records database, we did not observe a decreased risk of bladder cancer with use of itraconazole, a hedgehog pathway antagonist. These results could be explained if itraconazole's anti-tumor activity is dose or duration dependent, or if Hh signaling is lost during bladder tumorigenesis. Itraconazole is approved as an antifungal agent for oral doses ranging from 200 to 600 mg/day[20], over a relatively short treatment period. However, as observed in some studies, higher doses or an extended course of treatment may be required to inhibit Hh signaling for the purpose of cancer prevention[6,21].

Although bladder carcinoma is derived from Hh expressing cells in the basal layer of the bladder lumen, loss of Hh expression during progression to invasive bladder cancer has been recently described. Shin et al. reported that loss of Hh expression or genetic ablation of Hh signaling reduces induction of stromal differentiation factors, resulting in increased numbers of undifferentiated urothelial cells with invasive behavior, ultimately leading to invasive carcinoma [5]. Furthermore, some studies have suggested an association between pharmacologic blockade of Hh signaling and dramatically accelerated cancer progression in other cancers, such as pancreatic cancer [22,23,24]. Our finding of an increased risk of invasive bladder cancer requiring cystectomy with itraconazole use provides support, on a population-level, to these pre-clinical data.

There are several important strengths of this study. To our knowledge, this is the first population-based assessment of the Hh inhibitor, itraconazole, on bladder cancer risk and progression. The dataset used in this study, THIN, has been previously validated for the outcome of bladder cancer [15]. Likewise, detailed prescription information is captured in THIN as the electronic medical record is used to generate prescriptions for the patient.

Another unique feature of this study is the comparison of itraconazole to other azoles, which reduced the potential for confounding by indication. Because itraconazole is the only azole to suppress Hh signaling, we hypothesized that among azoles, an association with bladder cancer, if present, would be unique to itraconazole. Although we observed no significant association between itraconazole or other azoles and bladder cancer risk, it appeared that other azole users had slightly increased bladder cancer risk while itraconazole users had

lower risk. Furthermore, treatment with itraconazole but not the other azoles was associated with increased risk of invasive bladder cancer requiring cystectomy.

There were several potential limitations of this study. As these data indicate, most exposure to itraconazole was short-term (median duration of therapy 28 days) at recommended doses (200–600mg/daily). Therefore, we were unable to study bladder cancer risk or progression with higher daily doses or longer duration of itraconazole therapy. With rare exceptions, long-term exposure to environmental factors such as smoking is generally required to cause or reduce the risk of cancer. As such, short-term use of intraconazole would not be expected to influence the risk of bladder cancer development. However, a pattern such as that observed in this study (i.e., increased risk of more invasive bladder cancer requiring cystectomy) could occur if itraconazole acts as a tumor promoter even if exposure does not influence the formation of de novo bladder cancer. In this model, itraconazole could shorten the time between tumor formation, invasion, and subsequent cystectomy. Possible explanations for the absence of a duration-response include limited numbers of cases and controls in the duration subcategories, or, a threshold-effect.

Itraconazole is among most potent Hh antagonist identified in a screen of 2400 commercially available drugs[6]. However, other potent antagonists exist including the chemotherapeutic agents vinblastine, vincristine, vinorelbine, and paclitaxel. Data on chemotherapy receipt or type is not available in the THIN dataset. Fortunately, these therapies are not standard treatments for either non-muscle-invasive or muscle-invasive bladder cancer. Additionally, THIN does not contain information on some variables known to affect both bladder cancer risk (race, occupational history, and family history) and progression (smoking heaviness, stage, and histology). These variables are not likely to be associated with azole use and therefore unlikely to account for residual confounding in this study.

Because cancer stage is not readily available in the database, we cannot be sure that all cases treated with cystectomy were in fact muscle-invasive [15]. In general, cystectomy is reserved for muscle-invasive disease and a small subset of non-muscle invasive tumors considered to be high risk for progression [25]. The positive predictive value (PPV) for muscle-invasion using THIN's codes for cystectomy is 70% (95CI, 60–80%)[15]. This corresponds to a 30% chance of misclassifying non-muscle-invasive tumors as muscle-invasive. Importantly, this misclassification is most likely not related to azole exposure (i.e., non-differential misclassification bias). Such bias, even if present, would result in an underestimation of the measured association between itraconazole exposure and bladder cancer progression defined by cystectomy (i.e, the true odds ratio for muscle-invasion with itraconazole treatment would be even greater than that observed)[26].

Unlike other azoles such as fluconazole, itraconazole is indicated primarily for the treatment of onychomycoses, blastomycosis, histoplasmosis, and aspergillosis fungal infections [27]. As such, the use of itraconazole may be more prevalent in immunosuppressed patients, and this patient population may have higher incidence of invasive bladder cancer requiring cystectomy[28]. However, post-hoc analyses adjusting for immunosuppressive diagnoses and

treatments did not appreciably change the two-fold increased risk of invasive cancer requiring cystectomy with itraconazole exposure.

As with all observational studies, there is the risk of unmeasured confounding. However, an unmeasured confounder would need to be strongly associated with both itraconazole treatment and the risk of aggressive bladder cancer to have biased the association to the results that we observed. Such factors are not readily apparent.

CONCLUSION

In summary, there was no association between itraconazole use and bladder cancer risk overall. However, there was an increased risk of invasive bladder cancer requiring cystectomy with intraconazole treatment. These data provide clinical evidence supporting the role of Hh signaling in regulating human bladder cancer progression. However, as an observational study, this study cannot prove causality and is not sufficient to change clinical recommendations regarding choice of antifungal drugs. Further studies are needed to confirm our findings, assess for a dose and duration response, and clarify underlying biological mechanism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Drs. Mamtani and Boursi had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of data analysis. Drs. Mamtani and Boursi contributed to the conception and design of the study, and acquired the data; Drs. Scott, Yang, Lewis, Boursi and Mamtani contributed to analysis and interpretation of the data, drafting the article or revising it critically for important intellectual content, and final approval of the version to be published. Dr. Boursi would like to thank the Djerassi family for supporting his post-doctoral fellowship.

Funding: This research was supported by the National Institutes of Health (grant number K23 CA187185 to RM, K08-DK095951-02 to FIS and K24-DK078228 to JDL).

Abbreviations

Hh	Hedgehog
THIN	The Health Improvement Network

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Table 1

Characteristics of bladder cancer cases and control subjects

	Cases (n=13,440)	Controls (n=52,421)
Age at index date, median, y (IQR)	73.2 (64.9–80.1)	72.8 (64.5–79.9)
Male sex (%)	9,792 (72.8)	38,170 (72.8)
Duration of follow-up, median, y (IQR) [a]	6.0 (3.0–9.0)	6.0 (3.0–9.0)
Cigarette smoking history (%)		
Never	5,768 (42.9)	29,665 (56.6)
Ever	7,672 (57.1)	22,756 (43.4)
Recurrent bladder infections (%) [b]	1,466 (10.9)	2,414 (4.6)
Diabetes mellitus (%)	1,836 (13.7)	5,626 (10.7)
Obesity [BMI ≥ 30 kg/m^2] (%)	2,611 (19.4)	9,160 (17.5)
Chronic NSAID or ASA use (%)[c]	2683 (20%)	9,441 (18.0)
Metformin use (%)	849 (6.3)	2,702 (5.2)
Insulin use (%)	256 (1.9)	669 (1.3)
Use of other diabetes drugs (%)	711 (5.3)	2068 (3.9)
Itraconazole use (%)	86 (0.6)	354 (0.7)
Duration of therapy, median, w (IQR)	4.0 (2.0–4.3)	4.0 (1.1–5.0)
Use of other azoles (%)	686 (5.1)	2,368(4.5)
Duration of therapy, median, w (IQR)	8.0 (4.0–14.0)	6.0 (4.0–12.0)
Prior cancer[d]	1,021 (7.6)	2,306 (4.4)

SD, standard deviation; y, years; NSAID, non-steroidal anti-inflammatory drugs; w, weeks; IQR, inter-quartile range.

[a] Before index date

[b] More than 2 urinary tract infections

[c] Cumulative duration of therapy more than 1 year

[d] Prior history of breast, lung, colon, or prostate cancer

Table 2

Association of treatment with itraconazole and other azoles compared to no azole treatment and risk of bladder cancer (cases=13,440, controls=52,421)

	Cases N (%)	Controls N (%)	Unadjusted OR (95% CI)	Adjusted OR [a] (95% CI)
Never exposed to an azole	12,668	49,699	Reference	Reference
Ever exposed to itraconazole [b]	86 (0.7)	354 (0.7)	0.97 (0.76–1.23)	0.89 (0.70–1.14)
Number of itraconazole prescriptions				
1 course	56 (0.4)	210(0.4)	1.07 (0.79–1.44)	0.96 (0.71–1.30)
2–3 courses	21 (0.2)	102 (0.2)	0.81 (0.50–1.29)	0.75 (0.46–1.22)
≥4 courses	9 (0.1)	42 (0.1)	0.84 (0.41–1.72)	0.87 (0.42–1.81)
Ever exposed to other azoles [b]	686 (5.1)	2,368 (4.6)	1.15 (1.05–1.26)	1.06 (0.97–1.16)
Number of other azole prescriptions				
1 course	340 (2.5)	1233 (2.4)	1.09 (0.97–1.24)	1.01 (0.89–1.14)
2–3 courses	195 (1.5)	653 (1.3)	1.18 (1.00–1.39)	1.10 (0.93–1.30)
≥4 courses	151 (1.1)	482 (0.9)	1.25 (1.04–1.51)	1.15 (0.95–1.39)

[a] Conditional logistic regression adjusted for smoking (ever vs never), obesity (body mass index ≥30 kg/m^2), diabetes mellitus, use of diabetes medications (metformin, insulin, or thiazolidinediones), chronic use of aspirin or non-steroidal anti-inflammatory drugs (>1 year), and recurrent bladder infections.

[b] Receipt of at least one prescription for itraconazole or other azoles at least one year before the index date

Table 3

Association of itraconazole treatment compared to other azoles and risk of bladder cancer (cases=772, controls=2,722)

	Cases N (%)	Controls N (%)	Adjusted OR [a] (95% CI)	Fully-Adjusted OR [b] (95% CI)
Ever exposed to other azoles [c]	686	2368	Reference	Reference
Ever exposed to itraconazole [c]	86 (11.1)	354 (13.0)	0.84 (0.65–1.08)	0.84 (0.65–1.09)
Number of itraconazole prescriptions				
1 course	56 (7.2)	210(7.7)	0.97 (0.71–1.34)	0.96 (0.70–1.33)
2–3 courses	21 (2.7)	102 (3.7)	0.75 (0.46–1.22)	0.75 (0.46–1.22)
> 4 courses	9 (1.2)	42 (1.6)	0.78 (0.38–1.62)	0.84 (0.40–1.75)

[a] Logistic regression adjusted for age, sex, calendar period, and duration of follow-up.

[b] Logistic regression adjusted for age, sex, calendar period, duration of follow-up, and smoking (ever vs never), obesity (body mass index ≥30), diabetes mellitus, use of diabetes medications (metformin, insulin, or thiazolidinediones), chronic use of aspirin or nonsteroidal anti-inflammatory drugs (>1 year), and recurrent bladder infections.

[c] Receipt of at least one prescription for itraconazole or other azoles at least one year before the index date

Table 4

Association of treatment with itraconazole and other azoles compared to no azole treatment and risk of bladder cancer requiring cystectomy among patients diagnosed with bladder cancer (cases=1,004, controls=12,436)

	Cases N (%)	Controls N (%)	Adjusted OR [a] (95% CI)	Fully-Adjusted OR [b] (95% CI)
Never exposed to an azole	948	11,720	Reference	Reference
Ever exposed to itraconazole [c]	13 (1.3)	73 (0.6)	2.04 (1.11–3.74)	2.05 (1.12–3.78)
Number of itraconazole prescriptions				
1 course	8 (0.8)	48 (0.4)	1.96 (0.91–4.21)	1.90 (0.88–4.11)
≥2 courses	5 (0.5)	25 (0.2)	2.18 (0.81–5.84)	2.35 (0.88–6.28)
Ever exposed to other azoles [c]	43 (4.3)	643 (5.2)	0.84 (0.61–1.16)	0.83 (0.60–1.15)
Number of other azole prescriptions				
1 course	21 (2.1)	319 (2.6)	0.83 (0.53–1.31)	0.82 (0.52–1.29)
≥2 courses	22 (2.2)	324 (2.6)	0.85 (0.55–1.33)	0.84 (0.54–1.31)

[a] Logistic regression adjusted for age, sex, calendar period, and duration of follow-up

[b] Logistic regression adjusted for age, sex, calendar period, duration of follow-up, and smoking (ever vs never), obesity (body mass index ≥30), diabetes mellitus, use of diabetes medications (metformin, insulin, or thiazolidinediones), chronic use of aspirin or nonsteroidal anti-inflammatory drugs (>1 year), and recurrent bladder infections.

[c] Receipt of at least one prescription for itraconazole or other azoles at least one year before the index date

Table 5

Association of itraconazole treatment compared to other azoles and risk of bladder cancer requiring cystectomy among patients diagnosed with bladder cancer (cases=56, controls=716)

	Cases N (%)	Controls N (%)	Adjusted OR [a] (95% CI)	Fully-Adjusted OR [b] (95% CI)
Ever exposed to other azoles [c]	43	643	Reference	Reference
Ever exposed to itraconazole [c]	13 (23.2)	73 (10.2)	2.16 (1.07–4.36)	2.30 (1.12–4.72)
Number of itraconazole prescriptions				
1 course	8 (14.3)	48 (6.7)	2.18 (0.88–5.38)	2.25 (0.90–5.64)
≥2 courses	5 (8.9)	25 (3.5)	2.28 (0.74–7.00)	2.43 (0.76–7.80)

[a] Logistic regression adjusted for age, sex, calendar period, and duration of follow-up.

[b] Logistic regression adjusted for age, sex, calendar period duration of follow-up, and smoking (ever vs never), obesity (body mass index (≥30), diabetes mellitus, use of diabetes medications (metformin, insulin, or thiazolidinediones), chronic use of aspirin or nonsteroidal anti-inflammatory drugs (>1 year), and recurrent bladder infections.

[c] Receipt of at least one prescription for itraconazole or other azoles at least one year before the index date