NON-INTERVENTIONAL (NI) STUDY REPORT

PASS information

Title	Evaluation of the potential association between voriconazole use and squamous cell carcinoma (SCC) of the skin among patients with lung or lung/heart transplants
Protocol number	A1501097
Version identifier	1.0
Date	Final 4 December, 2015
EU Post Authorisation Study (PAS) register number	ENCEPP/SDPP/5269
Active substance	Voriconazole (ATC: J02AC03)
Medicinal product	Vfend®
Procedure number	EMEA/H/C/000387/FUM Post-Authorisation Measure MEA 071.11
Marketing Authorisation Holder (MAH)	Pfizer Limited Ramsgate Road, Sandwich, Kent CT130NJ United Kingdom
Joint PASS	No

Research question and objectives	**Primary objective:** To assess the potential association between voriconazole use and the development of SCC of the skin in patients with lung or heart/lung transplant. **Secondary objective:** To assess the potential association between voriconazole use and the development of melanoma in patients with lung or heart/lung transplant.
Country(-ies) of study	Retrospective data were collected from lung transplant centres from the following countries: • Australia • Canada • France • Germany • Italy • The Netherlands • Spain • Switzerland • United States of America
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1. ABSTRACT

Background

Voriconazole (Vfend®) is approved for the treatment of invasive aspergillosis (IA) and other invasive fungal infections (IFI). Voriconazole was also approved in June 2014 for prophylaxis of IFI in high-risk recipients of hematopoietic stem cell transplantations (HSCT) in the European Union. In addition to the approved indications, published reports indicate that voriconazole is commonly used as prophylaxis to prevent IA in solid organ transplant (SOT) recipients, primarily in lung or lung/heart transplant (LT) recipients.

Based on published case reports and retrospective observational studies, concerns have been raised about the risk of squamous cell carcinoma (SCC) of the skin with voriconazole administration, particularly in patients with immunocompromised status such as patients with solid organ transplant (SOT). The incidence of SCC of the skin is reported to be 65 to 250 times higher in SOT recipients than in the general population.

An observational study was conducted to evaluate the risk of SCC of the skin associated with voriconazole exposure in patients with LT. This non-interventional study was designated as a Post-Authorisation Safety Study (PASS) and was a commitment to the European Medicines Agency (EMA).

Objectives

a. Primary objective

To assess the potential association between voriconazole use and the development of SCC of the skin in patients with lung or heart/lung transplant.

b. Secondary objective

To assess the potential association between voriconazole use and the development of melanoma in patients with lung or heart/lung transplant.

Methods

A retrospective cohort study using secondary data sources was designed to address the objectives. Lung or lung/heart transplant recipients constituted the study population. Consecutive patients aged ≥ 18 years that underwent LT between 1 January, 2005 and 31 December, 2008 were identified from 14 LT centres in the EU, North America and Australia.

Study eligible patients were followed from the date of LT to whichever of the following occurred first:

- Occurrence of SCC of the skin or melanoma (for patients developing one endpoint (e.g., SCC), follow up was continued for the occurrence of a second endpoint (e.g., melanoma)
- Death
- Last patient visit or 31 December, 2012, after which all surviving patients not experiencing an outcome were censored.

Patient-level data on voriconazole utilization, biopsy-confirmed diagnosis of SCC of the skin and melanoma, demographic and clinical characteristics including immunosuppressive agents, and underlying diseases at the time of LT were collected from each patient's medical record and entered into an electronic database.

Cumulative (not necessarily consecutive) voriconazole exposure of at least 30 days was considered clinically meaningful for the main analyses; the same criterion was applied to other azoles. Exposure to voriconazole and other azoles (i.e., posaconazole, fluconazole and itraconazole) were considered as time-dependent variables.

At each post-LT time point, an individual patient could be in any one of the following four exposure categories:

1. Totally unexposed to any azole, or exposed to some azole for less than 30 days (hereafter referred to as ***"unexposed"***),

2. Exposed to voriconazole for 30 days or longer but not to any other azole for 30 days or longer (hereafter referred to as ***"exposure to voriconazole alone"***)

3. Exposed to other azole(s) for 30 days or longer but not to voriconazole for 30 days or longer (hereafter referred to as ***"exposure to other azoles alone"***), *or*

4. Exposed to voriconazole for 30 days or longer as well as exposed to some other azole for 30 days or longer (hereafter referred to as ***"exposure to voriconazole and other azole(s)"***).

A patient's follow-up time spent in any of these four exposure status was allocated to the corresponding person-time category. Consequently, a patient may have contributed person-time in more than one exposure categories.

Incidence rates (number of events per 1000 person-years) of SCC of the skin and melanoma were estimated by the four treatment exposure categories. A multivariate Cox regression analyses was conducted to assess the risk of SCC of the skin with voriconazole exposure controlling for confounding variables including age, gender, history of malignancy pre-LT, confounding by indication (by using underlying diseases), patients' immune status and immune intensity (by using immunosuppressive agents and mean cyclosporine and tacrolimus levels), and exposure to sun light (by using geographical location of transplant centres by latitude).

Results

A total of 900 consecutive patients aged ≥ 18 years undergoing LT were included in the final analysis dataset: 440 (48.9%) from 7 centres in the EU, 430 (47.8%) from 6 centres in North America and 30 (3.3%) from one centre in Australia. More than half of the study patients were males and older than 50 years of age. Patients with ≥ 1 day voriconazole exposure and no exposure to voriconazole were comparable with regards to age and gender.

Incidence rate of SCC of the skin by treatment exposure

Overall, the incidence rate (per 1,000 person-years) of SCC of the skin was 33.4 in the exposure to voriconazole alone category, 10.4 in the exposure to other azoles alone category, 21.7 in the exposure to voriconazole and other azole(s) category, and 13.1 in the unexposed category.

Multivariable analyses to assess the risk of SCC of the skin with voriconazole

In a multivariable Cox regression model analyzing voriconazole, other azoles and immunosuppressive agents as time-dependent variables, exposure to voriconazole alone (adjusted hazard ratio [HR]=2.39, 95% CI: 1.31-4.37) and exposure to voriconazole and other azole(s) (adjusted HR=3.45, 95% CI: 1.07-11.06) were associated with SCC of the skin as compared with the unexposed after controlling for the confounding variables.

Effect of duration of voriconazole exposure on the risk of SC of the skin

A separate multivariable model adjusting for potential confounders suggested gradual increase in the risk with increasing cumulative duration of voriconazole exposure. Compared with no exposure to any azole, cumulative voriconazole exposure of 91-180 days (adjusted HR=2.23, 95% CI: 0.94-5.30) and >180 days (adjusted HR=3.52, 95% CI: 1.59-7.79) showed a higher risk of SCC of the skin.

Effect of dose of voriconazole exposure (measured as DDD) on the risk of SCC of the skin

A multivariable model adjusting for potential confounders suggested that an increase of one defined daily dose (1 DDD = 400 mg daily) in mean daily exposure to voriconazole increased the risk of SCC of the skin by 2.70-fold (adjusted HR=2.70, 95% CI: 1.53-4.78).

Among the 900 patients, one case of melanoma was identified— a male, aged 39 years from The Netherlands. The patient had undergone double LT secondary to interstitial pulmonary fibrosis (IPF) and was not exposed to any azole.

Conclusions

The study data suggest a 2.39-fold increased risk associated with exposure to voriconazole alone for 30 days or longer and a 3.45-fold increased risk associated with exposure to

voriconazole and other azole(s) each for 30 days or longer compared with unexposed category. Of 900 patients included in the study, 55 developed SCC of the skin. Overall, median time from LT to the diagnosis of SCC of the skin was 3.3 years (range: 0.23 - 6.7 years). The study attempted to control for all major confounding variables and biases. Voriconazole and other azoles were analyzed as time-dependent variables to account for time-varying exposures. Attempt was made to control for potential confounding by immunosuppression by inclusion of immunosuppressive agents and mean cyclosporine and tacrolimus levels in the final multivariable model. Underlying diseases and transplant rejection episodes were included in the multivariate model to control for confounding by indication. It is well known that it is often impossible to obtain sufficiently accurate estimate of the effect of confounding by indication since "indication" is a complex and multifactorial phenomenon. Although data on underlying diseases, mean levels of cyclosporine and tacrolimus, and transplant rejection episode were used in the multivariable model in an attempt to control for potential confounding by indication, residual confounding by indication cannot be ruled out in this study.

Given that only one case of melanoma was identified among the study patients, analysis using inferential statistics for this endpoint was not possible.

The primary objective of this PASS was to evaluate the risk of SCC of the skin with voriconazole exposure and the study did not assess the effectiveness of voriconazole treatment such as reduction in IA or IFIs and/or all-cause mortality in this special patient population. It is important to carefully weigh the risk of SCC of the skin and benefits of voriconazole when prescribing to patients with LT.

2. LIST OF ABBREVIATIONS

Abbreviation	Definition
BCC	Basal Cell Carcinoma
COPD	Chronic Obstructive Pulmonary Disease
CMV	Cytomegalovirus
CI	Confidence Interval
CNI	Calcineurin Inhibitor
DDD	Defined Daily Dose
EMA	European Medicines Agency
EU	European Union
EMR	Electronic Medical Records
HR	Hazards Ratio
HSCT	Hematopoietic Stem Cell Transplantation
HPV	Human Papillomavirus
HIV	Human Immunodeficiency Virus
IA	Invasive Aspergillosis
IDSA	Infectious Diseases Society of America
IPF	Interstitial Pulmonary Fibrosis
ICU	Intensive Care Unit
IRB/EC	Institutional Review Board/Ethics Committee
IFI	Invasive Fungal Infection
IQR	Interquartile Range

NMSC	Non-Melanoma Skin Cancer
LT	Lung or Lung/Heart Transplant
PASS	Post Authorisation Safety Study
RR	Relative Risk
SAP	Statistical Analysis Plan
SCC	Squamous Cell Carcinomas
SOT	Solid Organ Transplant
UK	United Kingdom
US	United States of America

3. PRINCIPAL INVESTIGATOR(S) OF THE PROTOCOL

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4. MILESTONES

Milestone	Planned date *DD/Month/YYY*	Actual date *DD/Month/YYY*	Comments
Registration in the EU PAS register	Before staring data collection	26 November, 2013	
Start of data collection		2 December, 2013	
End of data collection	31 December, 2014	31 December, 2014	
Study progress report-I	6 Sept 2013	29 August, 2013	
Study progress report-II	28 April 2014	18 April, 2014	
Study progress report-III	28 April 2015	26 February, 2015	
Final report of study results	30 September, 2015	The MAH has notified the EMA that the planned final study report submission dated 30th September, 2015 (which was 9 months after completion of data collection) cannot be met because of unanticipated complexities in the data analyses (Communication submitted 18th August, 2015). The MAH has committed to submit the final study report within 12-month of end of data collection in accordance with Guideline on Good Pharmacovigilance practices (GVP) module VIII Post-Authorisation Safety Studies.	

5. RATIONALE AND BACKGROUND

Voriconazole (Vfend®) is approved in the USA and Europe for the treatment of invasive aspergillosis (IA) and other invasive fungal infections (IFI) in 2002. Voriconazole has shown superior efficacy compared with other antifungal agents and is currently recommended as the drug of choice for IA by the Infectious Diseases Society of America (IDSA) [1]. Voriconazole was approved in June 2014 for prophylaxis of IFI in high-risk recipients of hematopoietic stem cell transplantations (HSCT) in the European Union. In addition to the approved indications, published reports indicate that voriconazole is used as prophylaxis to prevent IA in solid organ transplant (SOT) recipients, primarily in lung or lung/heart transplant (LT) recipients [1, 2].

Patients undergoing SOT including LT typically receive immunosuppressive agents for a period of time after the transplantation to prevent organ rejection. This prolonged immunosuppression renders these patients highly susceptible to invasive fungal infections (IFIs) such as IA [3]. As a result, antifungal prophylaxis is commonly prescribed for patients at the time of the transplant in many centres worldwide. The data from a worldwide survey showed that voriconazole is the preferred antifungal for prophylaxis either as monotherapy or in combination with another antifungal agent [4].

Squamous cell carcinoma (SCC) of the skin is the second most common skin cancer in the general population after basal cell carcinoma (BCC), and it is the most common cancer in immunocompromised patients with SOT. Overall, the incidence of SCC of the skin in SOT patients is reported to be 65 to 250 times that of the general population [5], and varies by type of organ transplant. Furthermore, SCC of the skin has been reported to be more aggressive and has been associated with a high mortality in immunocompromised patients [6]. The recognized risk factors for the development of SCC of the skin include prolonged sunlight exposure, long duration of immunosuppressive therapy, infection with human papillomavirus (HPV) and lower CD4 cell counts, and certain host factors such as male gender, old age, White race and Fitzpatrick skin types I, II, or III [7-9].

Single case reports [10-12] and small case series [13-15] of SCC of the skin have been reported in patients with immunocompromised status treated with voriconazole such as patients with SOT, hematological malignancy or infection with human immunodeficiency virus (HIV). Recently, the risk of SCC of the skin or non-melanoma skin cancer (NMSC) with voriconazole administration has been investigated in observational analytical studies, primarily among recipients of LT [16-21] but also in HSCT recipients [22].[1] All the studies were retrospective and use patient-level data collected from the respective transplant centre however the findings were not generally consistent. This may be due in part to the difference in exposure/endpoint assessment and/or analytical methods employed by the investigators.

[1] It is to be noted that only two analytical studies (Vadnerkar, 2010 and Feist, 2012) investigating the risk of the SCC with voriconazole were published at the time of the study protocol development

Further, the majority of the published studies did not adequately account for the immunosuppressive agents received by transplant patients when assessing the potential association between voriconazole use and SCC of the skin. Transplant patients who were treated with voriconazole, particularly for long durations (e.g., >180 days), might be more immunocompromised compared with patients who were not treated with voriconazole or who were treated for a short period of time. Therefore, the effect estimates for SCC of the skin or NMSC in some studies may have been confounded by the immune status of the patients.

Another important consideration, which was not assessed in most of the published studies investigating the risk of SCC of the skin with voriconazole exposure, is the potential for confounding by indication. Given that voriconazole is the preferred antifungal agent for prophylaxis either as monotherapy or in combination with other antifungal agents, as shown in a worldwide survey of SOT centres [4], it is likely that physicians would use voriconazole if patients appear to be severely ill/immunocompromised. This could result in a spurious association of voriconazole use with untoward outcomes, including the development of SCC of the skin. In addition, some of these studies did not control for the presence of co-morbid/underlying conditions and/or use of concomitant medications and therefore, confounding by indication in these studies cannot be ruled out. Given the limitations of the currently available published data, the independent contribution of voriconazole to the development of SCC of the skin, a multifactorial outcome, could not be thoroughly assessed. Therefore, to better understand the risk of SCC of the skin in patients receiving voriconazole a well-designed, scientifically robust study is needed that should account for important confounding factors, particularly immunosuppressive agents, when assessing the potential association between voriconazole use and the development of SCC of the skin.

In addition to the reports of SCC of the skin, there are reports of melanoma in immunocompromised patients receiving prolonged voriconazole therapy [23]. To date, there have been no published analytical studies that have investigated the association between voriconazole exposure and the development of melanoma.

Based on the comprehensive study feasibility assessment of existing EU and US databases conducted by the Marketing Authorisation Holder (MAH), it was concluded that an adequately powered retrospective cohort study in the 'overall voriconazole-treated population' would not be feasible because of the low incidence of SCC of the skin.[2] However, the background incidence of SCC of the skin has been reported to be relatively high in the LT patient population, and the majority of cases of SCC of the skin reported in patients receiving voriconazole have been in patients with LT. This non-interventional, retrospective cohort study aimed to evaluate the association between voriconazole use and the development of SCC of the skin in patients with LT using real-world data.

[2] Feasibility Assessment Report submitted to the EMA on 20 September, 2010

This study was designated as a Post-Authorisation Safety Study (PASS) and was a commitment to the European Medicines Agency (EMA).

6. RESEARCH QUESTION AND OBJECTIVES

6.1. Primary objective

- To assess the potential association between voriconazole use and the development of SCC of the skin in patients with lung or heart/lung transplant.

6.2. Secondary objective

- To assess the potential association between voriconazole use and the development of melanoma in patients with lung or heart/lung transplant.

7. PROTOCOL AMENDMENTS AND UPDATES

No major amendment was made to the protocol. The protocol included an overview of the proposed statistical analyses. The statistical analysis plan (SAP) was refined and expanded to comprehensively analyse the study as described in 8.12.4 'Amendments to the SAP.

8. RESEARCH METHODS

8.1. Study design

This was a retrospective cohort study.

8.2. Setting

Lung or lung/heart transplant recipients constituted the study population. Study eligible patients were identified from a multicentre, multinational database of patients with LT. The study database collected retrospective patient-level data from 14 LT centres in the EU, North America and Australia (described in detail in 9.7 Data Sources and Measurements).

8.3. Subjects

Consecutive study eligible patients that underwent LT at the participating study centres between 1 January, 2005 and 31 December, 2008 were included.

8.4. Inclusion and exclusion criteria

Inclusion criteria

- Patient aged ≥ 18 years at the time of LT
- Patient received LT between 1 January, 2005 and 31 December, 2008

Exclusion criteria

- Patient with simultaneous or sequential abdominal organ transplant

8.5. Definition of voriconazole and other azole exposure

The protocol categorized study patients into two cohorts 1) voriconazole exposed cohort: patients with LT who received ≥ 1 dose of voriconazole regardless of whether they also received other antifungals, and 2) unexposed cohort: Patients with LT who did not receive voriconazole

However, preliminary review of study data showed that a substantial proportion of patients (a) received other azoles i.e., posaconazole, fluconazole, itraconazole as well (switched from/to voriconazole), or (b) did not receive any azole (including voriconazole) during the follow up period. Therefore, to appropriately analyze the complex data, exposure to voriconazole and other azoles were analysed as time-dependent variables and measured as person-time of exposure.

Cumulative (not necessarily consecutive) voriconazole exposure of at least 30 days was considered clinically meaningful for the main analyses; the same criterion was applied to other azoles.

At each post-LT time point, an individual patient could be in any one of the following four exposure categories:

1. Totally unexposed to any azole or exposed to some azole for less than 30 days (hereafter referred to as ***"unexposed")***,

2. Exposed to voriconazole for 30 days or longer but not to any other azole for 30 days or longer (hereafter referred to as ***"exposure to voriconazole alone****")*,

3. Exposed to other azole(s) for 30 days or longer but not to voriconazole for 30 days or longer (hereafter referred to as ***"exposure to other azoles alone****")*, *or*

4. Exposed to voriconazole for 30 days or longer as well as exposed to some other azole for 30 days or longer (hereafter referred to as ***"exposure to voriconazole and other azole(s)"***.

A patient's follow-up time spent in any of these four exposure status was allocated to the corresponding person-time category. Consequently, a patient may have contributed person-time in more than one exposure categories. As an example: for a patient who did not receive any azole for the first 90 days post-LT and was then treated with voriconazole for 60 days starting on day 91 post-LT, the patient would have contributed the first 90 days plus the initial 29 days on voriconazole to the "unexposed" category and from day 120 to the end of follow up to the "exposure to voriconazole alone" category. Figure II illustrates determination of treatment exposure categories at each time point during follow up using

hypothetical examples. The steps for defining start of "at risk period of clinically meaningful exposure (≥ 30 days) to an anti-fungal therapy are described in Appendix- I.

Although ≥ 30 days of cumulative exposure to voriconazole was considered clinically meaningful for assessing the risk of SCC of the skin, additional sensitivity analysis defining voriconazole exposure as ≥ 1 day was also conducted, as planned in the original SAP.

8.6. Index date and follow up

The index date was the "date of LT". The study eligible patients were followed from the index date to whichever of the following occurred first:

- Occurrence of SCC of the skin or melanoma (for patients developing one endpoint (e.g., SCC), follow up was continued for the occurrence of a second endpoint (e.g., melanoma)
- Death
- Last patient visit based on documentation in medical records or 31 December, 2012, after which all surviving patients not experiencing an endpoint were censored.

8.7. Variables

8.7.1. Exposure

The following data on voriconazole use were collected from medical records:

- Dose
- Duration of therapy (this was calculated by using the treatment start and stop dates)
- Reason for voriconazole use (for prophylaxis, treatment or both)

Data on antifungal agents other than voriconazole (i.e., itraconazole, posaconazole, amphotericin B) were also collected. In addition, data on antifungals use prior to LT was collected, when available in medical records.

8.7.2. Outcomes

The primary and secondary outcomes of the study were defined as the first occurrence of SCC of the skin or melanoma respectively during the follow up (i.e., observation) period. Data on the biopsy-confirmed diagnosis of SCC of the skin and melanoma were obtained from medical records. In addition, data on phototoxic reactions were collected when documented in medical records.

8.7.3. Risk factors for SCC of the skin including potential confounders and effect modifiers

In addition to the exposure and outcomes variables, data on the following risk factors including potential confounders and effect modifiers for the SCC of the skin were collected, as available in medical records:

- Age

- Gender
- Race/ethnicity
- Occupation
- Geographical location
- History of immune disorder prior to LT
- Malignancy prior to LT
- Type of transplant (lung only, double lung, heart-lung, re-transplant)
- Cytomegalovirus (CMV) serostatus (donor and recipient)
- Reason for transplant (e.g., chronic obstructive pulmonary disease (COPD), cystic fibrosis, interstitial pulmonary fibrosis, sarcoidosis, other)
- Days in intensive care unit (ICU) at the time of LT
- Days in hospital at the time of LT
- Hemodialysis within 30 days post LT
- Number of transplant rejection episodes
- Number of episodes of neutropenia after LT
- Use of immunosuppressive agents post LT during the follow up
  - Interleukin (IL)-2 inhibitors (i.e., basiliximab, daclizumab, alemtuzumab).
  - Alemtuzumab (Campath)
  - Thymoglobulin
  - Calcineurin inhibitors (CNIs) (tacrolimus, cyclosporine) – with number of episodes with elevated levels
  - Sirolimus, everolimus
  - Mycophenolate
  - Azathioprine
  - Corticosteroids
- Use of potentially phototoxic agents post LT
- Presence or absence of phototoxic reactions after the LT as documented in medical records
- A diagnosis of SCC and/or melanoma prior to LT

8.8. Data sources and measurements

Study eligible patients were identified from a multicentre, multinational database, which was developed at the University Health Network/University of Toronto, Canada.[3] This database collected retrospective patient-level data from 14 lung transplant centres in the EU, North America and Australia that agreed to contribute patient-level data to the database. The data was collected from centres with 'complete electronic medical records' (EMR) (i.e.,

[3] The feasibility assessment of existing solid organ transplant registries in the EU conducted by the MAH showed that these registries do not contain data on antifungals use including voriconazole. Therefore, none of the existing registries in SOT recipients could be used to investigate the risk of SCC with voriconazole administration among patients with LT. Subsequently, the MAH collaborated with the Principal Investigator based at the University of Toronto, Canada to develop a new database by compiling patient-level data from transplant centres in EU, North American and Australia.

maintaining all medical records such as medication use, diagnosis, pathology reports in both inpatient and outpatient settings in EMR) and 'partial EMR'(i.e., some data in EMR and others in paper- records). Data collection from transplant centres with EMR systems helped to minimize the time needed to compile the database.

Twenty two LT centres were invited to participate in the study (11 from across Europe, 7 from North America, and 4 from Australia), as follows:

- 14 centres, listed below (also in Table 2), agreed to participate in the study and contributed the data (7 from Europe, 6 from North America and 1 from Australia).

1. Hanover Medical School, Hannover, Germany
2. Lausanne-Geneva Lung transplantation program, Lausanne and Geneva, Switzerland
3. Ismett/Upmc, Palermo, Italy
4. University Medical Centre Groningen, Groningen, The Netherlands
5. Hospital Puerta De Hierro, Madrid, Spain
6. Hospital Universitario Y Politecnico La Fe, Valencia, Spain
7. Hôpital Européen Georges-Pompidou, Paris, France
8. University Health Network/University of Toronto, Canada (Principal study centre)
9. University of Texas Health Science Centre, San Antonio, US
10. University of Pennsylvania, Philadelphia, US
11. University of California, San Francisco, US
12. University of Southern California, Los Angeles, US
13. University of Pittsburgh Medical Centre, Pittsburgh, US
14. The Royal Adelaide Hospital, Adelaide, Australia.

- 8 centres declined or were considered not eligible to participate in the study. The reasons for exclusion included 1) centre declined or did not respond to the invitation, 2) patient data protection laws did not allow centre to participate, or 3) centre did not meet eligibility criterion of having an EMR to maintain patient records.

As recommended by the CHMP to obtain data from transplant centres in Australia,[4] all 4 LT centres in Australia (listed below) were invited to participate in the study.

1. The Alfred Hospital, Melbourne
2. The Prince Charles Hospital, Queensland
3. St. Vincent's Hospital, Sydney
4. The Royal Adelaide Hospital, Adelaide

Only one of the 4 centres 'The Royal Adelaide Hospital' agreed to participate and contributed the data whereas the other three centres declined to participate in the study.

[4]Outcome Fax: EMA/808209/2013 dated 20 December, 2013

Each of the 14 study centres provided patient-level data using the electronic case-report form (CRF) to the principal study centre 'University Health Network/University of Toronto, Canada'. The data were transferred in secure servers that transmit information only over encrypted connections. The electronic study database contained fields for all study variables with several check/validation variables to identify potential inconsistencies in data points for individual patient records. The database only contained the encrypted identification of the study patients and was protected by a firewall and a password.

8.9. Bias

This section describes potential sources of bias and efforts to assess and address them in this study.

- **Confounding by indication:** Voriconazole is a preferred antifungal agent in many transplant centres worldwide. Physicians are more likely to use voriconazole than other azole in severely ill patients at high risk for IFIs. This could result in a spurious association of voriconazole use with untoward outcomes including SCC of the skin. Data on underlying conditions and immunosuppressive agents were used in the multivariable analysis to control for this confounding by indication.

- **Misclassification of exposure**: The study data showed that approximately 68% of patients started receiving voriconazole within 30 days of LT, 20% started between 31 and 365 days, and 12% started voriconazole after 365 days post LT. Therefore, the span of time between cohort entry and the first prescription of voriconazole was "unexposed" in patients who did not start voriconazole on the day of LT. Start of "at risk period" at LT in those patients (as originally specified in the SAP) would have classified this unexposed time as exposed to voriconazole which might have resulted in an artificially lower rate of SCC of the skin among voriconazole exposed patients. Therefore, in order to prevent misclassification of exposure, time period from LT to exposure threshold for voriconazole or other azole in such patients was classified as unexposed in the analyses. It is to be noted that follow up of all patients started at the time of LT. However "at risk period" for patients exposed to voriconazole or other azole started after 30 days of cumulative exposure, whereas at risk period for unexposed patients started at the time of LT.

- **Confounding by immunosuppression:** Patients' immunocompromised status has been linked to the development of SCC of the skin. This study attempted to control for the confounding effect of immune status by including immunesuppressive agents and mean tacrolimus and cyclosporine levels in the multivariable model.

- **Time-dependent covariates**: Preliminary review of study data showed that a substantial proportion of patients (a) received other azoles as well (switched from/to voriconazole), or (b) did not receive any azole during follow up period. Therefore, to appropriately analyze this complex data, exposure to voriconazole and other azoles were analysed as time-dependent variables to account for changes in exposures (as described in Section 8.5 'Definition of voriconazole and other azole exposure').

8.10. Study Size

Sample size calculations were conducted at the time of protocol development to estimate the minimum number of patients needed to address the primary objective. Table 1 describes the sample size estimations of a retrospective cohort study addressing the study's primary objective based on different assumptions. Assuming a p_0 value (i.e., incidence of SCC of the skin in LT patients unexposed to voriconazole) of 5% and voriconazole exposed-to-unexposed ratio of 1:2, at least 157 patients in the voriconazole exposed cohort and 314 patients in the unexposed exposed cohort were needed to detect a relative risk (RR) of 2.5 with 80% power at a 5% significance level.

8.11. Data transformation

There was one final analysis dataset containing data from all 14 study centres. All analyses were conducted on de-identified data using STATA/MP 12.1. Any deviations to the SAP and/or additional analyses were documented in Section 8.12.4 'Amendments to the SAP'.

Variables transformation/categorization:

Below is the description of variables categorization (the reference category for univariate and multivariate analyses specified with [R])

- Age was categorized into the following categories: 18-29[R], 30-49, 50-59, 60-69, and ≥70 years
- Race was categorized into Caucasian, Hispanic, African-American, Asian and other[R]
- Occupation was categorized into indoors (e.g., office clerk, teacher, homemaker)[R], outdoors (e.g., driver, farmer, roofer), or both (e.g., unemployed, retired)
- Exposure to sunlight was categorized into low exposure to sunlight (>45° Latitude)[R], medium (35-45° Latitude), and high (<35° Latitude) based on the respective study centre's geographical location by Latitude.
- Immunosuppressive agents and immune score. Immunosuppressive agents are generally weaned over time (with occasional increases) in patients with LT. Therefore, each immunosuppressive agent was treated as a time-dependent variable to adequately control for confounding associated with immunosuppression, and categorized into[5]:

[5] Combined immunosuppressive agents are usually prescribed. Calcineurin inhibitors, including cyclosporine and tacrolimus have been the cornerstones of an immunosuppressive regimen, which usually includes 2 or more additional agents, almost always glucocorticoids, and a purine antagonist (mycophenolic acid or azathioprine). Sirolimus (rapamycin) has been used as a substitute for CNIs. The choice of agents is often immunosuppressive- protocol driven but is usually adapted to each recipient's risk profile or intolerance to one of these agents.

- Cyclosporine/Mycophenolate[R]
- Cyclosporine/Azathioprine
- Tacrolimus/Mycophenolate
- Tacrolimus/Azathioprine
- Rapamycin

Further, a patient immune scale that has been developed and used by other investigators to quantify the degree of immunosuppression in units/day was utilized as well. On this scale, 1 unit of immunosuppression was assigned to the corresponding doses of immunosuppressive agent mg/day: prednisone 5mg, azathioprine 100mg, cyclosporine 100mg, tacrolimus 2mg, mycophenolate mofetil 500mg, mycophenolate sodium 360mg, everolimus 1.5 mg, and sirolimus 2mg [25].

- The following variables were categorized into binary categories:
  - Immune disorder prior to LT (yes/no[R])
  - History of SCC prior to LT (yes/no[R])
  - Other malignancy prior to LT (yes/no[R])
  - Hemodialysis within 30 days of LT (yes/no[R])
  - Comorbid conditions e.g., Diabetes (yes/no[R])
- The following variables were categorized into multiple categories after examining the distribution:
  - Days in hospital at the time of LT (i.e., 1-14[R], 15-30,>30 days)
  - Number of transplant rejection episodes (i.e., 0[R], 1-2, 3-4, >4)
  - Number of neutropenia after LT (i.e., 0[R], 1-2, 3-4, >4)

8.12. Statistical methods

Detailed information on the statistical methods was documented in the Statistical Analysis Plan (SAP). Any deviations to the SAP and/or additional analyses are documented in 8.12.4 'Amendments to the SAP' of this report.

8.12.1. Main summary measures

Descriptive Measures

- Counts and frequencies for categorical variables
- Means for continuous variables

Given that a patient may have contributed to more than one exposure categories, presenting baseline demographic and clinical characteristics of study patients exposed to voriconazole or other azole for at least 30 days is not applicable. For the descriptive analysis, patients were categorized into two categories: ≥ 1 day exposure to voriconazole and no exposure to voriconazole.

Main Analysis Measures

- Unadjusted incidence rate of SCC of the skin (primary endpoint) and melanoma (secondary endpoint) over the total person-time of observation by four treatment exposure categories: unexposed, exposure to voriconazole alone, exposure to other azole alone, and exposure to voriconazole and other azole(s).

- Unadjusted hazard ratios (HRs) for SCC of the skin and melanoma

- Adjusted HRs for SCC of the skin and melanoma, adjusting for age, gender, immunosuppressive agents, mean cyclosporine level, mean tacrolimus level, sun exposure, history of malignancy pre-LT, transplant rejection episodes, underlying disease.

Exposure to voriconazole, other azole and immunosuppressive agents were analysed as time-dependent exposures (i.e., using person-time) for the main analysis.

8.12.2. Main Statistical Methods

Analytic Sample/Dataset

Analyses to address the primary and secondary objectives were conducted using all study patients eligible for the study.

Descriptive Statistics

Counts and frequencies for categorical variables including age at LT, gender , race/ethnicity, occupation, geographical location, occupation, reasons for LT, co-morbid conditions and immunosuppressive agents and mean for continuous variables (e.g., cyclosporine and tacrolimus levels).

As described earlier, for the descriptive analysis, patients were categorized into two groups: ≥ 1 day exposure to voriconazole and no exposure to voriconazole (Figure 1).

Incidence rates

For estimation of the incidence of SCC of the skin, exposure was categorized into four categories: unexposed, exposure to voriconazole alone, exposure to other azole alone, and exposure to voriconazole and other azole(s).

The incidence rate (per 1,000 person-years) of SCC of the skin was estimated using the following formula:

- *Incidence of SCC of the skin = (# of new cases of SCC of the skin / person-time at risk) x 1,000*
  (Patients with a history of SCC were excluded when estimating the incidence rate)

Person-time at risk

Exposure to voriconazole and other azoles (i.e., posaconazole, fluconazole and itraconazole) was analysed as time-dependent variables and classified into four treatment categories as described in Section 8.5 'Definition of voriconazole and other azole exposure'. A patient's follow-up time spent in any of these four exposure status was allocated to the corresponding person-time category. Consequently, a patient may have contributed person-time in more than one exposure categories.

All patients were followed from the date of LT to whichever of the following occurred first:

- Study outcomes i.e., diagnosis of SCC of the skin or melanoma (for all patients reaching one endpoint, follow up was continued for the occurrence of a second endpoint),
- Death,
- Last visit based on documentation in medical records, or
- 31 December, 2012, at which time all surviving patients not experiencing an outcome were censored.

A case of SCC of the skin was counted in a specific exposure category if the diagnosis was made at the time patient was classified in that particular exposure category. For example, if SCC of the skin was diagnosed at the time a patient was categorised as exposed to voriconazole alone, the event was counted in the numerator of exposure to voriconazole alone category for incidence rate estimation.

In addition, SCC of the skin incidence rate by four treatment exposure categories was calculated across the following demographic and clinical characteristics:

- Age
- Gender
- Occupation (i.e., indoors, outdoors, mixed indoors/outdoors; and exposed to chemical yes/no)
- Race/ethnicity
- Geographical location (i.e., study countries)
- Exposure to sunlight (i.e., high, medium and low)
- Type of LT (i.e., Single lung, double lung, lung-heart)
- Re-LT
- Underlying condition (e.g., COPD, cystic fibrosis, interstitial pulmonary fibrosis, primary pulmonary fibrosis)
- Immune disorder prior to LT
- History of other malignancy prior to LT
- Duration of hospitalization at the time of LT
- Duration of ICU admission at the time of LT
- Number of transplant rejection episodes
- Number of episodes of neutropenia after LT

- Co-morbid conditions (e.g. Diabetes)
- Hemodialysis dialysis within 30 days of LT
- CMV status (D-R-, D+R+, D-R+, D+R-)
- Immunosuppressive agents
- Immune score

In addition, SCC of the skin incidence rate was calculated by the duration of patient follow up from LT (e.g., incidence rate at year-1 post LT through incidence rate at year-7 post LT) across the four treatment exposure categories

In a separate analysis, SCC of the skin reoccurrence rate among patients with a history of SCC was calculated using the following formula:

[Number of patients with SCC recurrence/total number of patients with a history of SCC prior to LT]

It is to be noted that patients with a history of SCC were excluded from the main analyses.

Univariate analyses (Unadjusted analyses)

Univariate analysis was conducted to evaluate the association between voriconazole and SCC of the skin. Exposure to voriconazole, other azoles and immunosuppressive agents were analysed as time-dependent variables in the univariate analyses.

Further, exposure to voriconazole dose (measured as daily defined dose [DDD]) on the risk of SCC of the skin was also assessed. DDD was measured using the following formula from the World Health Organization (WHO) Collaborating Centre for Drug Statistics Methodology[6]:

[voriconazole cumulative dose in grams/voriconazole DDD 0.4 grams]

In addition to the treatment exposure categories, univariate association between other covariates and SCC of the skin was evaluated.

Multivariate analyses (Adjusted analyses)

A multivariable Cox proportional hazards regression model was developed to assess the independent effect of voriconazole exposure on the risk of SCC of the skin controlling for the effect of known and potential confounding variables. Exposure to voriconazole and other azoles were analyzed as time-varying covariates in the Cox model to take into account varying exposures to azoles during follow-up in this non-interventional study. For each case

[6] Source: World Health Organization <http://www.whocc.no/ddd/definition_and_general_considera/>

of SCC, the model utilized comparator patients who were still being followed at the time of SCC occurrence to prevent potential confounding by varying length of follow up, and considered their treatment exposure category as of that point of time in estimating the HR.

Variables with a p value of <0.2 in the univariate analyses were included in the final multivariable model. Known confounders (i.e., gender, transplant rejection episodes) were included in the final model regardless of results of the univariate analyses. Patients were censored the same way as the univariate analysis. A two-sided p-value of <.05 were considered statistically significant in all analyses.

The multivariable model adjusted for the following covariates:

- Age
- Gender
- Exposure to sunlight
- Immunosuppressive agents (analysed as time-dependent variables)
- Number of transplant rejection episodes
- Mean cyclosporine level
- Mean tacrolimus level
- Transplant rejection episodes
- History of malignancy (other than skin) prior to LT
- Underlying diseases

Effect of dose of voriconazole exposure on the risk of SCC of the skin

The effect of dose of voriconazole as measured in DDD on the risk of SCC of the skin was evaluated in a separate multivariate Cox regression model adjusted for the above-listed potential confounders. Exposure to voriconazole was analysed as a time-dependent variable in this multivariate analyses.

Effect of cumulative duration of voriconazole exposure on the risk of SCC of the skin

A separate multivariable model was developed to examine the effect of cumulative duration (not necessarily consecutive) of voriconazole or other azole exposure (categorized as 1-90 days, 91-180 days, and >180 days) on the risk of SCC of the skin adjusted for the potential confounders. Exposure to voriconazole and other azole was analysed as a time-dependent variable in this multivariate analyses.

Sensitivity analysis

The multivariable model was repeated with a cut off of ≥ 1 day exposure (instead of ≥ 30 days for the main analysis) to voriconazole or other azoles to evaluate the association between four treatment exposures and SCC of the skin adjusting for all potential confounders.

Other analyses

The following two additional multivariable models adjusting for all potential confounders were developed to evaluate the association between voriconazole and SCC of the skin:

- Voriconazole as a binary exposure variable (≥ 30 days exposure to voriconazole *vs.* <30 days or no exposure to voriconazole) instead of four treatment exposure categories. Exposure to voriconazole was analysed as a time-dependent variable in this analysis.

- Voriconazole as a binary exposure variable (≥1 day exposure to voriconazole *vs.* no exposure to voriconazole) instead of four treatment exposure categories. Exposure to voriconazole was analysed as a time- dependent variable in this analysis as well.

8.12.3. Missing values

Data on immunosuppressive medications including dosing was available up to four years post-LT. For patients who had longer than four years of follow-up (28%), both exposure and dose of immunosuppressant until end of follow-up were carried forward. This was based on the assumption that the vast majority of patients are clinically stable with respect to the possibility of acute rejection and there is likely little change in immunosuppression after four years post LT.

All other variables included a category for missing values, where applicable.

8.12.4. Amendments to the statistical analysis plan (SAP)

Preliminary descriptive review of the collected study data revealed certain unanticipated characteristics of the observational data collected from real world clinical practice, which were not expected at the time of SAP development. Therefore, the planned analyses were modified and additional analysis added to appropriately and comprehensively analyze the data, which are summarized below:

- In the SAP, date of LT was specified as the start of 'at risk period' under the assumption that the majority of LT recipients will start receiving voriconazole at the time of LT. However, the collected data showed that approximately 68% of patients started voriconazole within 30 days of LT, 20% started between 31 and 365 days, and 12% started voriconazole after 365 days post LT. Therefore, such patients starting voriconazole after LT were not exposed to voriconazole for a considerable period following LT (i.e., accrued follow up time in the study also includes initial periods of non-exposure/non-risk). Start of "at risk period" at LT in those patients (as specified in the SAP) would have classified this unexposed time as exposed to voriconazole which might have resulted in an artificially lower rate of SCC of the skin among voriconazole exposed patients. To resolve this issue, exposure to voriconazole and other azole) was treated as a time-varying variable, as was described in the SAP. However, the 'at risk period' was modified to start at the time of first exposure to voriconazole (or other azole)

in those patients — and not at the time of LT. It is to be noted that follow up of all patients started at the time of LT. However "at risk period" for patients exposed to voriconazole or other azole started after 30 days of cumulative exposure, whereas at risk period for unexposed patients started at the time of LT.

- The protocol categorized study patients into two groups 1) voriconazole exposed group: "Patients with LT who received ≥ one dose of voriconazole regardless of receiving other antifungals", and 2) voriconazole unexposed group: "Patients with LT who did not receive voriconazole". However, preliminary review of study data revealed that a substantial proportion of patients (a) received other azoles as well (switched from/to voriconazole), or (b) did not receive any azole during the follow up period. Therefore, to appropriately analyze the complex data, exposure to voriconazole and other azoles were analysed as time-dependent variables to account for time-varying exposure. Cumulative, not necessarily consecutive, voriconazole exposure of at least 30 days, was considered clinically meaningful for the main analyses; the same criterion was applied to other azoles. At each post-LT time point, an individual patient could be in any one of the following four exposure categories: unexposed, exposure to voriconazole alone, exposure to other azole alone, and exposure to voriconazole and other azole(s).

- In the SAP, exposure to voriconazole was defined based on "at least one dose voriconazole". In this report, ≥ 30 days voriconazole exposure was considered clinically meaningful for the main analyses i.e., estimation of incidence of SCC of the skin and melanoma, and univariate and multivariate Cox regression analyses to assess the risk of SCC of the skin with voriconazole exposure. However, an additional sensitivity analysis based on ≥ 1 day exposure to voriconazole *vs.* no exposure to voriconazole (i.e., as originally planned in the SAP) was also conducted.

- Similar to defining ≥ 30 days exposure to voriconazole as clinically meaningful to assess the risk of SCC of the skin, exposure to other azole and immunosuppressive agents were also defined based on ≥ 30 days exposure for the univariate and multivariate analyses with a sensitivity analysis using ≥ 1 day of exposure.

- Imputation of missing data was not planned in the SAP. However, data on immunosuppressive agents including dosing was available up to four years post-LT in the dataset. For patients who had follow-up times longer than four years (28% [252/900]), both exposure and dose of immunosuppressive agents was carried forward until the end of follow-up. This was based on the assumption that the vast majority of patients are clinically stable with respect to the possibility of acute rejection and there is likely little change in immunosuppression after this period. All other variables included a category for missing values, where applicable, without imputation of data (i.e., as originally planned in the SAP).

8.13. Quality control

The Principal Investigator (PI) and his team followed the institutional guidelines of University Health Network/University of Toronto, Canada for data collection and

management. Data collected were periodically checked for consistency by the database manager. Internal quality checks of all collected data, analysis, and written materials were conducted. Quality review of the final analytic dataset, statistical analysis and study report were documented and retained by the PI and his team, as follows:

- Confirmed that the source of the data and/or results was documented and that results and data had been verified against the source
- Checked the internal consistency of any data presented in the document.
- Confirmed that the conclusions were accurate.

8.14. Protection of human subjects

Subject information and consent

This study utilized retrospective (i.e., existing data from medical charts) de-identified data with appropriate IRB/EC approvals. Therefore, informed consent from each patient was not required in this study.

Confidentiality of the data

Confidentiality of the data was maintained at all times. The database only contains encrypted identification of the patients and all analyses were conducted on the de-identified data and reported only in aggregate form. The database was protected by a firewall, and stored in a password protected computer.

Independent Ethics Committee (IEC)/ Institutional Review Board (IRB)

The final protocol, any amendments, and informed consent documentation were reviewed and approved by an Institutional Review Board(s) (IRB) and/or Independent Ethics Committee(s) (IEC) for each site participating in the study.

Ethical conduct of the study

The study was conducted in accordance with legal and regulatory requirements, as well as with scientific purpose, value and rigor, and followed generally accepted research practices described in Good Pharmacoepidemiology Practices (GPP) issued by the International Society for Pharmacoepidemiology (ISPE), European Medicines Agency (EMA), European Network of Centres for Pharmacoepidemiology and Pharmacovigilance (ENCePP) Guide on Methodological Standards in Pharmacoepidemiology, and Food and Drug Administration (FDA) Guidance for Industry: Good Pharmacovigilance and Pharmacoepidemiologic Assessment, FDA Draft Guidance for Industry and FDA Staff: Best Practices for Conducting and Reporting of Pharmacoepidemiologic Safety Studies Using Electronic Healthcare Data Sets.

9. RESULTS

Fourteen LT centres provided patient-level data for this observational study: 7 centres from Europe, 6 in North America and 1 from Australia. Of 14 centers, 7 used EMR only and 7 used a combination of EMR and paper records.

9.1. Participants

A total of 921 patients aged ≥ 18 years who received consecutive LT between 1 January, 2005 and 31 December, 2008 were initially identified at the 14 participating transplant centres. Eight patients with simultaneous or sequential abdominal organ transplant were excluded. Additionally, 13 patients with a history of SCC of the skin were also excluded from the main analysis i.e., estimation of the incidence rate, univariate and multivariate analyses to assess the risk of SCC of the skin with voriconazole exposure.
Data on 900 patients were utilized to address the primary and secondary study objectives. Median follow up time for all study patients (n=900) from LT to the diagnosis of SCC of the skin, death or end of study was 3.51 years (ranged from 1 day to 7.97 years) (data not shown in tables). No patient was lost to follow up since per protocol patients were censored at last visit based on documentation in medical records.

9.2. Descriptive data

As described earlier, for the descriptive analysis, treatment exposure was categorized into two groups: ≥ 1 day exposure to voriconazole (n=472) and no exposure to voriconazole (n=428).

Of 472 patients with ≥ 1 day exposure to voriconazole, 299 (63%) received voriconazole for prophylaxis, 133 (28%) received for treatment and 39 (8%) received for both prophylaxis and treatment. Data on the indication of use of voriconazole (e.g., prophylaxis or treatment) was not available for 3 (0.64%) patients[7].

Table 3 summarizes patients' demographic and clinical characteristics including the use of immunosuppressive medications. Below is a summary of patient characteristics:

- **Demographic characteristics by voriconazole exposure (≥ 1 day exposure to voriconazole *vs.* no exposure to voriconazole)**

[7] Following definitions/criteria was used to categorized voriconazole prophylaxis or treatment:
Prophylaxis: the antifungal is given when there is a negative culture.

i. Primary prophylaxis: indication against IFIs. If there was a negative culture for invasive organisms or if there was a positive culture pre-transplant.
ii. Secondary prophylaxis: indication against IFIs. Given in case of a positive culture, but there is no evidence of infection.

Treatment: If positive culture with clinical signs, or in case of suspicion of infection without positive culture confirmation.

More than half (> 50%) of study patients were males and older than 50 years of age. Patients with ≥ 1 day exposure to voriconazole and no exposure to voriconazole were comparable with regards to age and gender. 55.7% with ≥ 1 day exposure to voriconazole and 54.7% with no exposure to voriconazole had missing/unknown data on race. Of the remaining patients with information on race, the majority (41.5%) with ≥ 1 day exposure to voriconazole and 42.5% with no exposure to voriconazole were Caucasians. About 7.6% of patients with ≥ 1 day exposure to voriconazole had an occupation recorded as 'outdoors' compared to 4.9% with no exposure to voriconazole. Overall, slightly higher proportions of patients with ≥ 1 day exposure to voriconazole than no exposure to voriconazole were classified in medium and high exposure to sunlight (medium sun exposure: 59.3% *vs.* 54.9%; high sun exposure 7.0% *vs.* 4.9%) (Table 3).

Of 900 patients included in the study, 440 (48.9%) were from EU, 430 (47.8%) from North America and 30 (3.3%) from Australia. Twenty six patients were included from Italy but none of them were exposed to voriconazole.

- **Clinical characteristics by voriconazole exposure (≥ 1 day of exposure to voriconazole *vs.* no exposure to voriconazole)**

The majority (n=711) of the patients received bilateral LT: 80.3% patients with ≥ 1 day voriconazole exposure compared to 77.6% with no exposure to voriconazole. Reasons for LT between patients with ≥ 1 day exposure to voriconazole *vs.* no exposure to voriconazole included COPD (25.0% *vs.* 32.7%), interstitial pulmonary fibrosis (25.6 *vs.* 23.1) and cystic fibrosis (28.0%. *vs.* 16.1%). The majority (95.3% [858/900]) of the study patients had their first LT: 95.1% patients with ≥ 1 day exposure to voriconazole compared to 95.6% with no exposure to voriconazole.

Duration of hospital stay at the time of LT was comparable between patients with ≥ 1 day exposure to voriconazole and patients with no exposure to voriconazole. However, of patients with information on the duration of ICU admission at the time of LT, longer ICU stay was observed among patients with ≥ 1 day exposure to voriconazole than patients with no exposure to voriconazole: 15-30 days (13.1% *vs.* 4.4%), >30 days (22.2% *vs.* 9.6%). Overall, 27 (3.0%) had dialysis within 30 days of LT: 2.8% of patients with ≥ 1 day exposure to voriconazole compared to 3.3% with no exposure to voriconazole.

Of 900 patients, CMV serostatus among donor and recipients was negative in 22.2% patients with ≥ 1 day exposure to voriconazole and 19.6% in patients with no exposure to voriconazole whereas CMV serostatus was positive in both donor and recipients in 30.1% of patients with ≥ 1 day of exposure to voriconazole and 26.2% in patients with no exposure to voriconazole. A small number of patients (n=15) had an underlying immune disorder (e.g., RA, SLE, psoriasis) prior to LT: 1.5% in patients with ≥ 1 day exposure to voriconazole compared to 1.9% in patients with no exposure to voriconazole.

Overall, 560 (62.2%) patients experienced neutropenia after LT during the follow up period: 66.7% patients with ≥ 1 day exposure to voriconazole and 57.2% in patients with no exposure to voriconazole. Among patients who experienced neutropenia, the number of

episodes of neutropenia was generally comparable, except a higher proportion of patients had >4 episodes in patients with ≥ 1 day exposure to voriconazole compared to no exposure to voriconazole (21.2% *vs.* 9.8%). Two patients had malignancies other than SCC prior to LT – both patients had no exposure to voriconazole. None of the 900 patients that were included to address the primary and secondary study objectives had been diagnosed with SCC prior to LT.

Approximately, 50% of the patients experienced ≥1 transplant rejection episode. The number of transplant rejection episodes was generally comparable in both groups, except a higher proportion of patients had >4 rejection episodes in patients with ≥ 1 day exposure to voriconazole than in patients with no exposure to voriconazole (6.1% *vs.* 2.6%) (Table 3).

- **Use of immunosuppressive medications by voriconazole exposure (≥ 1 day exposure to voriconazole *vs.* no exposure to voriconazole)**

Compared to patients with no exposure to voriconazole, a higher proportion of patients with ≥ 1 day exposure to voriconazole received thymoglobulin (5.7% vs. 2.3%), alemtuzumab (26.7% *vs*. 0) and a lower proportion of patients received basiliximab (22.2% *vs.* 44.9%) induction post LT. Only a small number of patients (n=6) received daclizumab induction in the study.

A higher proportion of patients with ≥ 1 day exposure to voriconazole than no exposure to voriconazole received at least one dose of tacrolimus (80.7% *vs.* 59.6%), prednisone/methylprednisone (99.8% *vs.* 97.4%), mycophenolate (sodium/mofetil) (86.2% *vs.* 67.5%), sirolimus (10.8% *vs.* 3.5%), whereas a lower proportion of patients with ≥ 1 day voriconazole exposure than no exposure to voriconazole received ≥ 1 day of cyclosporine (50.6% *vs.* 65.2%), azathioprine (29% *vs.* 46.7%), and everolimus (6.4% *vs*. 8.6%). The distribution of immunosuppression score categories was comparable between the patients with ≥ 1 day exposure to voriconazole and patients with no exposure to voriconazole (Table 3).

Mean tacrolimus level was 9.6 (SD ±4.3) in patients with ≥ 1 day exposure to voriconazole compared to 8.1 ± 5.0 in patients with no exposure to voriconazole. Mean cyclosporine level was 207.8 (SD ± 135.5 in patients with ≥ 1 day exposure to voriconazole compared to 276.7 (SD ± 185.1) in patients with no exposure to voriconazole (data not shown in tables).

- **Use of potentially phototoxic medications by voriconazole exposure (≥ 1 day of exposure to voriconazole *vs.* no exposure to voriconazole)**

A total of 228 (25.3%) patients received potentially phototoxic medications (i.e., doxycycline, diltiazem, glyburide, hydroxychloroquine, isotretinoin, naproxen,and/or nifedipine, piroxicam) during the follow up period with a slightly higher proportion in patients with ≥ 1 day exposure to voriconazole than patients with no exposure to voriconazole (26.9% *vs.* 23.6%) (Table 3).

9.3. Outcome data

The analysis of outcome variable was conducted by four treatment exposure categories: unexposed, exposure to voriconazole alone, exposure to other azole alone, and exposure to voriconazole and other azole(s).

Primary outcome: SCC of the skin by four treatment exposure categories

Of 900 patients included in the study, 55 developed SCC of the skin. Overall, median time from LT to the diagnosis of SCC of the skin was 3.3 years (ranged: 0.23 - 6.7 years, 25th percentile 1.8 years; 75th percentile 4.2 years.

- Twenty eight cases of SCC of the skin were identified in the exposure to voriconazole alone category. Median time from voriconazole exposure to the diagnosis of SCC of the skin was 2.91 years (ranged: 0.29-2.94 years, 25th percentile 1.63 years; 75th percentile 3.45 years).

- Five cases of SCC of the skin were identified in the exposure to voriconazole and other azole(s) category. Median time from voriconazole (or other azole) exposure to the diagnosis of SCC of the skin was 3.73 years (ranged: 0.54-3.79 years, 25th percentile 2.86 years; 75th percentile 3.75 years).

- Five cases of SCC of the skin were identified in the exposure to other azole alone. Median time from the other azole exposure to the diagnosis of SCC of the skin was 2.41 years (ranged: 1.72- 5.90 years, 25th percentile 1.96 years; 75th percentile 3.94 years).

- Seventeen cases of SCC of the skin were identified in the unexposed category. Median time from LT to the diagnosis of SCC of the skin was 3.1 years (ranged: 0.23 years- 6.7 years, 25th percentile 2.4 years; 75th percentile 5.1 years).

Secondary outcome: Melanoma

Among the 900 patients, one case of melanoma was identified in a male aged 39 years, from The Netherlands. The patient had undergone double LT secondary to IPF and was not exposed to any azole. Time from LT to the development of melanoma was 3.6 years (data not shown in tables).

Other outcome: Phototoxic reactions by four treatment exposure categories:

Of 900 patients included in the study, 15 had phototoxic reactions recorded in the medical records. Eleven cases of phototoxic reactions were identified in the exposure to voriconazole alone category and 4 cases in the exposure to voriconazole and other azole(s) category. No cases of phototoxic reactions were identified in the exposure to other azole alone category and in unexposed category (data not shown in tables).

Of 55 patients with a diagnosis of SCC of the skin, 5 (9.1%) patients were identified with phototoxic reactions compared to 10 (1.2%) patients in 845 without SCC of the skin (data not shown in tables).

9.4. Main results

The incidence rate was estimated across the four treatment exposure categories: unexposed, exposure to voriconazole alone, exposure to other azole alone, and exposure to voriconazole and other azole(s). Voriconazole and other azole(s) exposures were analysed as time-dependent variables.

- **Incidence of SCC of the skin by four treatment exposure categories:**

Table 4 shows the crude incidence rate of SCC of the skin by the four treatment exposure categories. The incidence rate (per 1,000 person-years) of SCC of the skin was 33.4 in exposure to voriconazole alone, 10.4 in exposure to other azole alone, 21.7 in exposure to voriconazole and other azole(s), and 13.1 in unexposed. Overall, a gradual increase in the incidence rate was observed with increase in time since LT across all four treatment exposure categories .

The incidence was also estimated across various demographic and clinical characteristics. Within the exposure to voriconazole alone category, the incidence rate of SCC of the skin (per 1,000 person-years) was 48.2 in patients aged ≥60 years, 27.7 in patients aged 50-59 years, 46.6 in patients aged 30-49 years and 5.6 in patients aged 18-29 years. A higher incidence rate was noted among males than females across the treatment exposure categories, except in exposure to voriconazole and other azole(s) where the incidence was higher among females. Also, the incidence was higher among patients whose occupation required spending the majority of time outdoors compared with those with indoors occupation across the treatment exposure categories, except in exposure to voriconazole and other azole(s) where the incidence was observed to be higher in patients with indoors occupation.

SCC reoccurrence rate: Of 921 patients initially identified for this study, 13 patients had a history of SCC prior to LT. One of these 13 patients was diagnosed with a recurrent SCC yielding a recurrence rate of 7.7% (data not shown in tables). Note: As described in Section 8 Research Methods, patients with a history of SCC were excluded from the primary analysis.

- **Univariate analysis evaluating the risk of SCC of the skin with voriconazole exposure and other variables**

Table 5 shows results of the univariate analysis evaluating the association between SCC of the skin and exposure to voriconazole and other potential confounders.

Exposure to voriconazole, other azole(s) and immunosuppressive agents were analysed as time-dependent variables in the univariate analyses.

At the univariate level, exposure to voriconazole alone showed an increased risk for SCC of the skin compared with unexposed (HR=2.55, 95% CI: 1.42-4.60).

An increasing risk of SCC of the skin was observed with increasing age. Compared to 18-29 years, age groups ≥ 60 years (HR=15.04, 95% CI: 2.05-110.08) and 50-59 years (HR=9.22, 95% CI: 1.25-68.20) were associated with higher risk of SCC of the skin. A 'dose response relationship' was observed between exposure to sunlight and SCC of the skin. Exposures to medium sunlight (HR=3.37, 95% CI: 1.42-8.0) and high sunlight (HR=4.40, 95% CI: 3.50-23.49) were at higher risk for SCC of the skin compared with low sunlight exposure. A history of malignancy prior to LT was also associated with SCC of the skin (HR=22.06, 95% CI: 9.97-48.81). With regard to immunosuppressive agents, exposure to alemtuzumab (HR=2.44, 95% CI: 1.23-4.80), cyclosporine/azathioprine (HR=7.11, 95% CI: 1.56-32.50 and tacrolimus/mycophenolate (HR=4.35, 95% CI: 1.00-18.99) were significantly associated with the risk of SCC of the skin at the univariate level.

Gender, geographical location, occupation, exposure to chemicals, LT type, re-LT, immune disorder prior to LT, underlying conditions for LT, dialysis within 30 days post LT, transplant rejection episodes, neutropenia episodes, diabetes post LT, CMV serostatus, days in hospital at the time of LT, days in ICU at the time of LT and exposure to tacrolimus, steroids, mycophenolate, azathioprine, sirolimus, or everolimus were not statistically significantly associated with SCC of the skin at the univariate level.

- **Multivariate analysis evaluating the risk of SCC of the skin with voriconazole exposure controlling for potential confounders**

In a multivariable Cox regression model analyzing voriconazole, other azoles and immunosuppressive agents as time-dependent variables, exposure to voriconazole alone (adjusted HR=2.39, 95% CI: 1.31-4.37) and exposure to voriconazole and other azole(s) (adjusted HR=3.45, 95% CI: 1.07-11.06) compared with unexposed were associated with SCC of the skin after controlling for age, gender, history of malignancy pre-LT, underlying diseases, immunosuppressive agents, mean cyclosporine and tacrolimus levels, and exposure to sun light (Table 6).

Other covariates included in the multivariate model showing statistically significant association with SCC of the skin included ≥30 days treatment with cyclosporine/azathioprine compared to cyclosporine/mycophenolate (adjusted HR=6.48, 95% CI: 1.33-31.42), and exposure to high sunlight compared to low sunlight (adjusted HR=6.67, 95% CI: 2.29-19.41). Furthermore, underlying diseases at the time of LT, primary pulmonary hypertension (adjusted HR=5.08, 95% CI: 1.16-22.17) and scleroderma (adjusted HR=9.58, 95% CI: 1.56-58.79) compared to cystic fibrosis also showed statistically significant association with SCC of the skin (Table 6). Standard error estimates for the treatment exposures and other covariates included in the fully adjusted and final multivariable model remained robust throughout the multivariable modeling process. Further, the data did not suggest evidence of effect modification by variables of gender or age (P >0.10 for all tested interactions).

Effect of duration of voriconazole exposure on the risk of SCC of the skin

A separate multivariable model adjusting for potential confounders, suggested a dose-response relationship between the cumulative duration of voriconazole exposure and the risk of SCC of the skin. Compared with no exposure to any azole, cumulative voriconazole exposure of 91-180 days (adjusted HR=2.23, 95% CI: 0.94-5.30) and >180 days (adjusted HR=3.52, 95% CI: 1.59-7.79) showed higher risk of SCC of the skin (Table 7). The model did not suggest increase risk of SCC with increasing dose of other azole(s) compared to no exposure to any azole.

Effect of dose of voriconazole exposure (measured as DDD) on the risk of SCC of the skin

Further, a multivariable model adjusting for potential confounders suggested that an increase of one DDD (1 DDD = 400mg daily) in the mean daily exposure to voriconazole increased the risk of SCC of the skin by 2.70-fold (adjusted HR=2.70, 95% CI: 1.53-4.78) (Table 8).

9.5. Other analyses

- The multivariable analyses (summarized in Table 6) were repeated with exposures defined as ≥ 1 day exposure to voriconazole and ≥ 1 day exposure to other azoles. The adjusted model did not show statistically significant association with SCC of the skin: ≥1 day of exposure to voriconazole (adjusted HR=1.83, 95% CI: 0.94-3.55), ≥1 day exposure to other azole (adjusted HR=0.59, 95% CI: 0.18-1.94), ≥1 day exposure to voriconazole and other azoles each (adjusted HR=2.15, 95% CI: 0.76-6.07) compared with no exposure to any azole (Table 9).

- A separate fully adjusted multivariable model with ≥ 30 days exposure to voriconazole compared with < 30 days exposure o voriconazole (adjusted HR=2.69, 95% CI: 1.59-4.55) showed an increased risk of SCC of the skin (Table 10).

  When this analysis was repeated with ≥ 1 day exposure to voriconazole *vs.* no exposure to voriconazole, the association with SCC of the skin remained statistically significant however the HR and corresponding 95% CIs slightly changed (adjusted HR=2.21, 95% CI: 1.31-3.72) (Table 11).

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9.6. Adverse events / adverse reactions

This study protocol required review of the patient medical chart and/or a narrative field in the dataset. Review of patient medical charts and narratives for specific attribution of SAEs to Pfizer drugs were not actively pursued. However, while the primary purpose of this study did not encompass assessment of drug-related effects in individuals, the reviewer might have identified an SAE with explicit attribution to a Pfizer drug via patient chart and/or narrative review (and with an identifiable reporter). Such SAEs must be reported to Pfizer or its representative for submission to regulatory authorities. Explicit attribution is not inferred by a temporal relationship between drug administration and an SAE but must be based on a

definite statement of causality by a healthcare provider linking drug administration to the SAE.

A serious adverse event (SAE) was defined as any untoward medical occurrence that at any dose results in death;

- is life-threatening (i.e., at immediate risk of death due to the event);
- requires inpatient hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability/incapacity; or
- is a congenital anomaly/birth defect.

Medical and scientific judgment was exercised in determining whether an event is an important medical event. An important medical event many not be immediately life-threatening and/or result in death or hospitalization. However, if it is determined that the event may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above, the important medical event should be reported as serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse. If there was a written notation in the medical chart/narrative field indicating that a physician attributed a serious adverse event to a Pfizer drug, the abstractor completed an SAE form within 24 hours of identification of the event and submitted to Pfizer Safety. Since patients are de-identified, such information did not include any patient or physician identifying information such as name, address, or phone number.

There were no SAEs identified in this study. However, 2 unrelated events (visual hallucinations and gastrointestinal symptoms –both assessed as non-serious) experienced by a 57 years old female from Switzerland were identified during the study period. Although the events were reported to Pfizer, they do not meet the definition of reportable SAEs. The reporting physician mentioned that “there was not a reasonable possibility that these events were related to voriconazole or to concomitant drugs”.

10. DISCUSSION

10.1. Key results

As described in the background section, increased risk of SCC of the skin or NMSC with voriconazole exposure among patients with LT or bone marrow transplant (BMT) has been suggested in published observational studies utilizing institutional-based retrospective data. This retrospective cohort study investigated the association between voriconazole exposure and SCC of the skin in patients with LT. Exposure to voriconazole alone was associated with a 2.39-fold increased risk and exposure to voriconazole and other azole(s) was associated with a 3.45-fold increased risk for SCC of the skin compared with unexposed. Analysis of cumulative duration of voriconazole exposure suggests that longer duration of voriconazole exposure is associated with an increasing risk of SCC of the skin—the risk increased to approximately 3.55-fold with voriconazole exposure >180 days compared to no

exposure to any azole. An increment of 1 DDD in the mean voriconazole dose (400mg daily) was associated with a 2.70 fold higher incidence of SCC of the skin.

The strengths of this study include the large number of patients (n=900) recruited from multiple LT centres from across EU, North America and Australia, and collection of detailed data on exposure, outcome and potential confounders from medical records. As recommended by the CHMP's Rapporteur and described in detail in Section 8.12.4 Amendment to the SAP, voriconazole, other azole and immunosuppressive agents were analyzed as time-dependent variables to account for time-varying exposures.

Potential confounding factors in evaluating the association between voriconazole exposure and SCC of the skin are patients' immune status, confounding by indication (i.e., channeling bias) and exposure to prolonged sun exposure. This study attempted to control for these major confounding variables, as follows:

- Patient's immunocompromised status has been linked to increase in the risk of SCC of the skin. The increased risk of SCC of the skin in SOT recipients, particularly among LT recipients, is primarily attributed to the prolonged exposure to immunosuppressive agents that are prescribed to prevent allograft rejection in these patients. This study attempted to assess and control for potential confounding by immunosuppression status through multiple approaches: a) collecting comprehensive data on the use of immunosuppressive agents post LT, b) estimating mean levels of cyclosporine and tacrolimus to assess immunosuppression intensity, and c) calculating an immune score for each patient. The multivariable model evaluating the risk of SCC of the skin with voriconazole exposure included immunosuppressive agent and mean cyclosporine and tacrolimus levels to control for confounding by immunosuppression and immunosuppression intensity[8]. In addition, transplant rejection episodes were controlled for in the final analyses as an additional proxy variable for severity of immunosuppression.

- Voriconazole is a preferred antifungal prophylaxis agent in many LT centres worldwide [4]. Physicians are likely to use voriconazole to prevent IFIs if a patient appears to be immunosuppressed or presented with multiple co-morbidities. This could result in a spurious association of voriconazole use with untoward outcomes including SCC of the skin. Although complete control of confounding by indication is challenging and may not be possible, data on underlying diseases, mean levels of cyclosporine and tacrolimus, and transplant rejection episode were used in the multivariable model to control for potential confounding by indication.

- Intense or prolonged exposure to sunlight has been identified as a risk factor for the SCC of the skin. Exposure to sunlight or use of sun protective measures (e.g., use of sunscreen with high sun protection factor, [SPF]) is not routinely documented in medical charts for patients with LT and therefore was not available in medical charts of all the patients at

[8] Immunosuppressive agents were used in the multivariable model instead of immune score. Because immune score was derived from immunosuppressive agents

the participating centres. In order to control for the confounding effect of exposure to sunlight, a proxy variable based on geographical location of study centre by latitude was developed (categorized as low, medium and high exposure to sunlight) and used in the multivariate analysis in the absence of patients-level data on exposure to sunlight.

As described above, this study attempted to effectively control for all major known confounding variables. However, possibility of residual confounding cannot be completely ruled out. Therefore, caution should be exercised when interpreting the data.

It is to be noted that data supporting a biological mechanism of the development of SCC of the skin with exposure to voriconazole are limited [21]. In published case reports, some patients first developed phototoxic reactions during treatment with voriconazole before being diagnosed with SCC of the skin. This led to a suggestion that adverse phototoxic dermatological reactions may serve as a precursor for SCC in patients undergoing prolonged treatment with voriconazole. In this study, overall, 1.7% (15/900) of the study patients had phototoxic reactions recorded in their medical charts. The proportion was higher among patients with SCC of the skin compared to patients without a diagnosis of SCC of the skin (9.1% [5/55] *vs*. 1.2% [10/845]). However, the rate of phototoxic reactions observed in this study should be interpreted with caution given that the study utilized retrospective data based on medical charts. It is reasonable to assume that physicians may not observe and/or routinely document in medical records incidences of phototoxic reactions like skin rash or redness. As such, it is possible that phototoxic reactions were under reported in this study. Because of these limitations, meaningful inferences from the data on phototoxic reactions cannot be drawn.

10.2. Limitations

In addition to inherent limitations of an observational study using retrospective data such as possible inaccuracies in medical records, this study was subject to the following design/data-related limitations:

- **Misclassification of exposure:** The data on use of medications including azole 3 months prior to LT was not available, which might have resulted in misclassification of exposure. For example, some patients might have been treated with ≥30 days voriconazole or other azole 3 months prior to LT but classified as unexposed to azole in this study. However, given the majority of patients start to receive azole at the time of LT or post LT, it is reasonable to assume that the proportion of patients who might have received voriconazole or other azole(s) 3 months before LT would have been very small. Therefore, the chance of misclassification of exposure (if any) is expected to be minimal.

- **Residual confounding:** Given that proxy variables were used to control for confounding (i.e., immunosuppressive agents in the absence of a comprehensive measure of immune status; geographical location/latitude of LT centre in the absence of individual-level data on exposure to sunlight in medical charts), residual confounding due to these factors cannot be completely ruled out. It is well known that it is often impossible to obtain sufficiently accurate estimate of the effect of confounding by indication since

"indication" is a complex and multifactorial phenomenon [27]. Although data on underlying diseases, mean levels of cyclosporine and tacrolimus, and transplant rejection episode were used in the multivariable model in an attempt to control for potential confounding by indication, residual confounding by indication cannot be ruled out in this study.

- **Unmeasured confounding:**

- **Missing data on race/ethnicity:** This study was not able to control for race/ethnicity because of >55% missing information about this variable. There is no evidence that voriconazole prescription or utilization differs across various racial/ethnic strata but if any disparities existed, race or ethnicity might have been a potential unmeasured confounder in this study.

- **Non-availability of data on smoking:** Cigarette smoking is an identified risk factor for SCC of the skin [26]. Potential confounding due to smoking was not controlled for in the final analysis because of non-availability of the data in the medical records.

- **Limited sample size to analyze centre-level data:** Incidence of the SCC of the skin was estimated for each participating LT centre. However, analysis using inferential statistics to address the primary study objective was not possible because of the limited number of patients recruited from each participating centre.

10.3. Interpretation

The final multivariable model adjusting for potential confounders suggest a 2.39-fold increased risk associated with exposure to voriconazole alone and a 3.45-fold increased risk associated with exposure to voriconazole and other azole(s)compared with unexposed. An increment of 1 DDD in the mean voriconazole daily dose (400mg daily) was associated with a 2.70 fold higher incidence of SCC of the skin. Further, analysis of cumulative duration of voriconazole exposure suggests a dose-response relationship with SCC of the skin—the risk increased to a 3.55-fold with voriconazole exposure >180 days compared with no exposure to any azole. This study attempted to control for the major confounding variables including patients' immune status, confounding by indication (i.e., channeling bias) and exposure to sunlight in the multivariate analysis.

The primary objective of this PASS was to evaluate the risk of SCC of the skin with voriconazole exposure, and the study did not assess the effectiveness of voriconazole treatment such as reduction in IA, IFIs and/or all-cause mortality in this patient population. It is important to carefully weigh the risk of SCC of the skin and benefits of voriconazole when prescribing to patients with LT.

10.4. Generalisability

This study included data from 14 LT centres in the EU, North America and US to assess the risk of SCC of the skin with voriconazole exposure in patients with LT. Therefore, the findings are likely to be generalizable to LT recipients receiving voriconazole. However,

caution should be exercised when generalizing the findings to more diverse populations (i.e. all voriconazole-treated patient populations) given that patients with LT are a special patient population with unique factors that make them more susceptible to SCC of the skin.

11. OTHER INFORMATION

Not Applicable

12. CONCLUSIONS

This retrospective cohort study investigated the association between voriconazole exposure and the risk of SCC of the skin and melanoma among patients with LT using real-world data from 14 LT centres across EU, North America and Australia. A total of 900 consecutive patients aged ≥ 18 years undergoing LT centres were included in the final analysis dataset: 440 (48.9%) from 7 centres in EU, 430 (47.8%) from 6 centres in North America and 30 (3.3%) from one centre in Australia.

The final multivariable Cox model suggested that exposure to voriconazole alone for 30 days or longer (adjusted HR=2.39, 95% CI: 1.31-4.37) and exposure to voriconazole and other azole(s) each for 30 days or longer (adjusted HR=3.45, 95% CI: 1.07-11.06) compared with unexposed were associated with SCC of the skin after controlling for age, gender, history of malignancy pre-LT, underlying diseases, immunosuppressive agents, mean cyclosporine and tacrolimus levels, and exposure to sun light. A separate multivariable model adjusting for the confounders suggested gradual increase in the risk of SCC of the skin with increasing cumulative duration of voriconazole exposure.

Given that only one case of melanoma was identified among the study patients, analysis using inferential statistics for this endpoint was not possible.

The results of this observational retrospective study should be interpreted with caution. The study attempted to control for all major confounding variables and biases. Exposure to voriconazole and other azole was analyzed as time-dependent variables to account for time-varying exposures. An attempt was made to control for potential confounding by immunosuppression by inclusion of use of immunosuppressive agent and mean cyclosporine and tacrolimus levels in the final multivariable model. Underlying diseases and transplant rejection episodes were included in the multivariate model to control for confounding by indication. It is well known that it is often impossible to obtain sufficiently accurate estimate of the effect of confounding by indication since “indication” is a complex and multifactorial phenomenon. Although data on underlying diseases, mean levels of cyclosporine and tacrolimus, and transplant rejection episode were used in the multivariable model in an attempt to control for potential confounding by indication, residual confounding by indication cannot be ruled out in this study.

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14. LIST OF SOURCE TABLES AND FIGURES

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Figure I. Eligibility in this study evaluating the association between voriconazole exposure and SCC of the skin and melanoma in patients with lung or lung/heart transplant, and categorization of treatment exposure categories

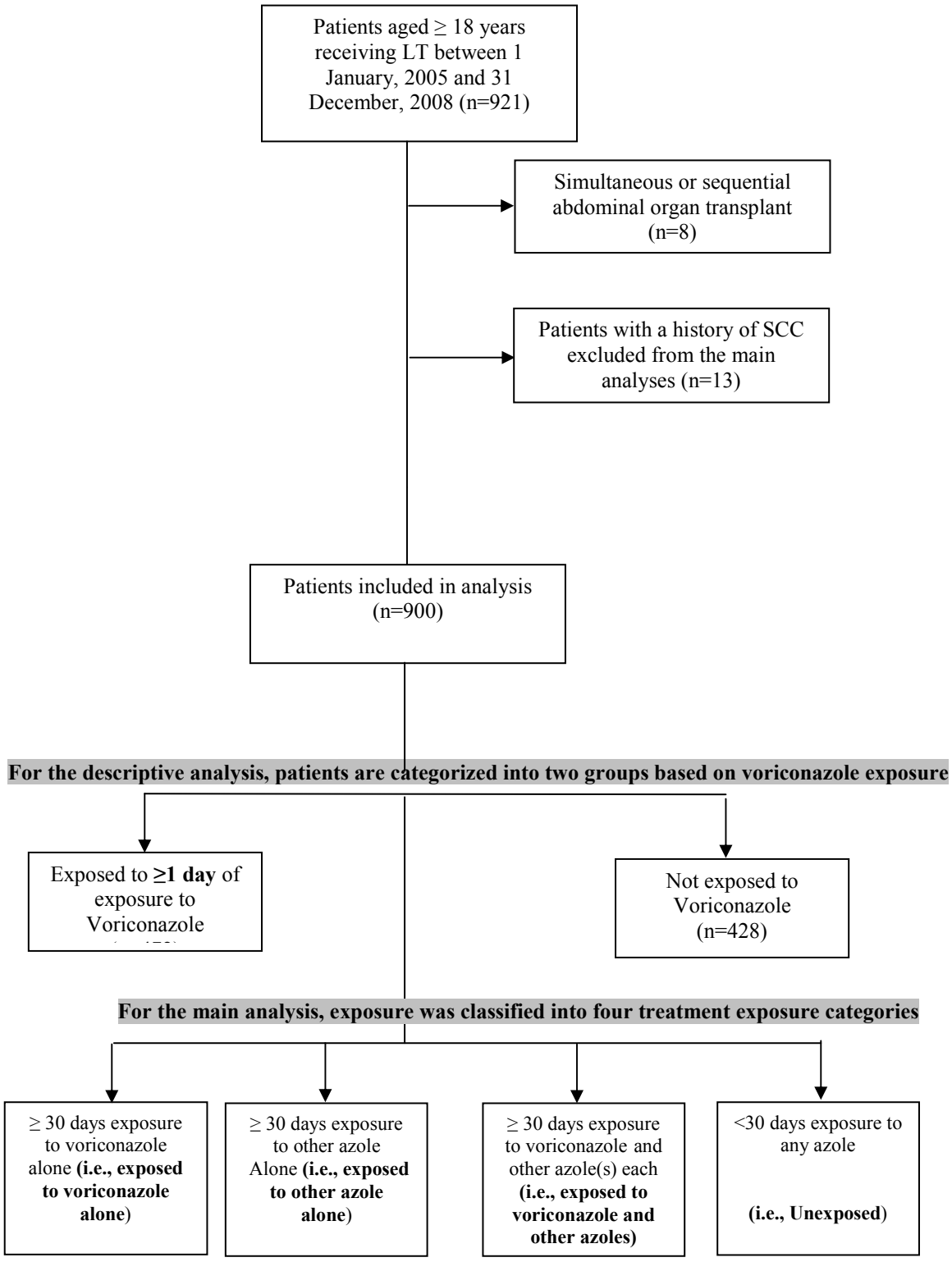
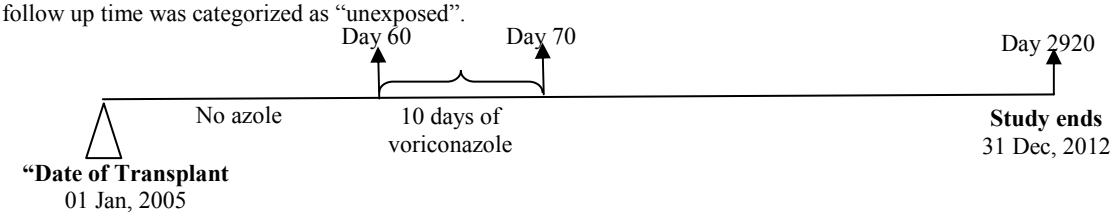


Figure II. Hypothetical examples of determination of treatment exposure categories at each time point during follow up

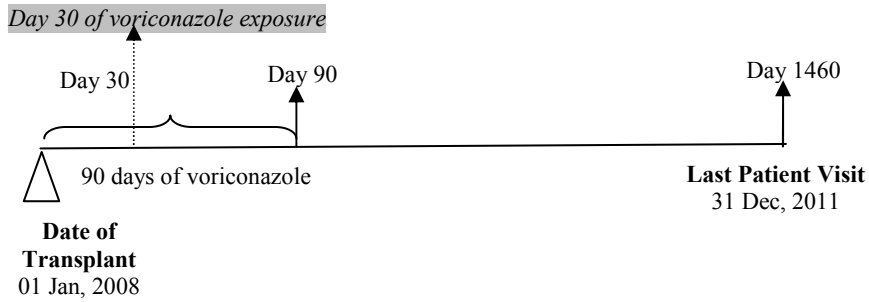
Definition of four treatment exposure categories:

- Totally unexposed to any azole or exposed to some azole for less than 30 days (**"unexposed"),**
- Exposed to voriconazole for 30 days or longer but not to any other azole for 30 days or longer (**"exposure to voriconazole alone**")
- Exposed to other azole(s) for 30 days or longer but not to voriconazole for 30 days or longer (**"exposure to other azoles alone**"), or
- Exposed to voriconazole for 30 days or longer as well as exposed to some other azole for 30 days or longer (**"exposure to voriconazole and other azole(s)"**

Example- I: Patient started receiving voriconazole at day 60 post-transplant and continued to receive for 10 days. Patient was alive on 31 Dec, 2012 (i.e., day 2920 post-transplant). Exposure to voriconazole was less than 30 days and therefore did not meet the criterion of clinically meaningful exposure. At each point from transplant to 31 Dec, 2012 the patient's follow up time was categorized as "unexposed".



Example –II: Patient started receiving voriconazole at the day of lung transplant and continued for 90 days. Patient's last visit based on medical records/end of follow up period was at day 1460 post-transplant. From the day of transplant to day 29, patient's follow time was categorized as "unexposed" because until that point exposure to voriconazole was less than 30 days and therefore did not meet the criterion of clinically meaningful exposure. In this example, exposure was categorized under voriconazole exposure alone even after discontinuing voriconazole at day 90 post-LT (from day 30 to the end of follow up at day 1460).



Example- III: Patient started receiving other azole at day 180 post-transplant and continued for 40 days, then switched to voriconazole at day 220 post-transplant and continued for 180 days (voriconazole stopped at day 400 post-transplant). Patient was diagnosed with SCC of the skin at day 1095 post-transplant. Follow up time from the transplant to day 209 is categorized as "unexposed" because until that point exposure to other azole was less than 30 days. Follow up time from day 210 to day 249 is categorized as "other azole alone". Time from day 250 to day 1095 (diagnosis of SCC/end of follow up) was categorized as "exposure to voriconazole and other azole" even after discontinuing voriconazole at day 400.

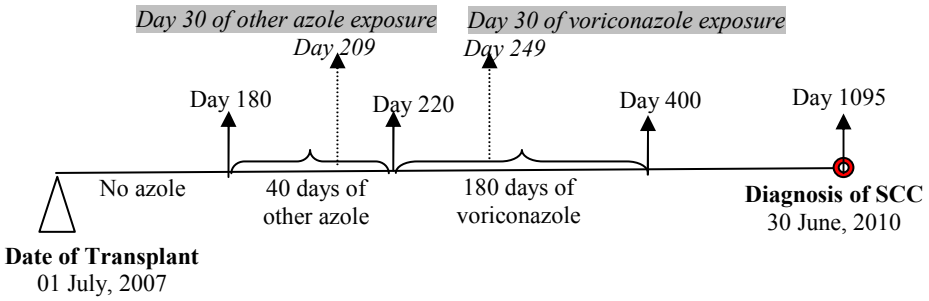


Table 1. Sample size estimation for a retrospective cohort study with a range of p_0, detectable relative risks (RRs), and voriconazole exposed-to-unexposed cohort ratios, with 80% power at a 5% significance level

The sample size calculations are based on the following assumptions:

- Two sided hypothesis tests with Alpha (α) level of 5%
- Power (i.e., the ability to statistically detect a difference between the two cohorts [i.e., voriconazole exposed cohort and unexposed cohort] when a statistical difference exists) is 80%.
- *Exposed-to- unexposed* (i.e., voriconazole exposed cohort-to- unexposed cohort) ratio ranges from 1:1, 1:2 to 1:3. The study feasibility assessment data suggest that there will be less number of patients with voriconazole exposure t than patients without voriconazole exposure. For sample size estimations, it is reasonable to assume that voriconazole exposed -to- unexposed ratio will range from 1:1, 1:2 to 1:3.
- Minimal detectable relative risk (RR) comparing voriconazole exposed cohort to unexposed cohort ranges from 2.0 to 3.0.
- Incidence of SCC of the skin in LT patients unexposed to voriconazole (p_0) ranges from 3.0% to 15.0%. In order to estimate the sample size for a cohort study, it is necessary to specify the value of p_0, the rate of SCC of the skin in patients unexposed to voriconazole, from the same source population (i.e., patients with LT) from which the voriconazole cohort will be drawn. The data on p_0 in the published literature is limited. Only one study was identified that reported an incidence of 9.9% for SCC and/or BCC of skin in patients with LT [28]. However, several published papers reported SCC of the skin incidence in similar patient populations (i.e., renal, heart, or liver transplant patients) [29-32].[9] For sample size calculations, it is reasonable to assume that the value of p_0 will range from 3.0% to 15.0%.

p_0	**RR to be detected**	**Minimum number of patients required in voriconazole exposed cohort and unexposed cohort for various *exposed-to-unexposed* ratios:**					
		1:1		**1:2**		**1:3**	
		Voriconazole Cohort	Non Voriconazole Cohort	Voriconazole Cohort	Non Voriconazole Cohort	Voriconazole Cohort	Non Voriconazole Cohort
3.0	2.0	749	749	536	1072	464	1392
	2.5	385	385	272	544	233	699
	3.0	245	245	171	342	146	438
4.0	2.0	552	552	396	792	343	1029
	2.5	283	283	200	400	172	516
	3.0	179	179	125	250	107	321
5.0	2.0	435	435	312	624	270	810
	2.5	222	222	157	314	135	405
	3.0	140	140	98	196	84	252

[9] The incidence of SCC of the skin in other organ transplant patients (i.e., other than lung or lung/heart transplant patients) is reported to range from 3.45% in patients with renal transplant patients in UK, 4.6% in patients with renal liver, heart, lung or pancreas transplant in Sweden, 12.6% in patients with liver transplant in the US, to 12.6% in patients with renal transplant in Spain.

6.0	2.0	356	356	256	512	222	666
	2.5	181	181	128	256	110	330
	3.0	114	114	80	160	68	204
8.0	2.0	258	258	185	370	161	483
	2.5	130	130	92	184	79	237
	3.0	81	81	57	114	49	147
10.0	2.0	199	199	143	286	124	372
	2.5	100	100	71	142	61	183
	3.0	62	62	44	88	37	111
13.0	2.0	145	145	104	208	91	273
	2.5	71	71	51	102	44	132
	3.0	43	43	31	62	27	81
15.0	2.0	120	120	87	174	76	228
	2.5	59	59	42	84	36	108
	3.0	35	35	25	50	22	66

Notes: Sample size was calculated using the method described by Dupont and Plummer (1990) and specifying an unmatched cohort design: Software implementing this method (PS power and sample size is available at: http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize

Assuming that the rate of p_0 is 5.0% and the voriconazole exposed–to unexposed cohort ratio is 1:2, the study would require at least 157 patients in the voriconazole exposed cohort and 314 patients in the unexposed cohort to detect a rate ratio of 2.5 with 80% power at a 5% significance level.

Table 2. Name of participating lung transplant centres, type of medical records keeping and the number of patients enrolled in the study

No.	Name	Type of Medical Records	Number of Patients
Transplant centres in Europe			
1.	Hanover Medical School, Germany	Complete EMR	195
2.	Lausanne-Geneva Lung transplantation program, Switzerland	Complete EMR	17
3.	Ismett/Upmc Palermo, Italy	Complete EMR	26
4.	University Medical Centre Groningen, The Netherlands	Complete EMR	91
5.	Hospital Puerta De Hierro, Madrid, Spain	Partial EMR	48
6.	Hospital Universitario Y Politecnico La Fe, Valencia, Spain	Partial EMR	36
7.	Hôpital Européen Georges-Pompidou, Paris, France	Partial EMR	27
Transplant centres in North America			
8	University of Texas Health Science Centre, San Antonio, US	Complete EMR	14
9.	University of Pennsylvania, Philadelphia, US	Partial EMR	10
10.	University of California, San Francisco, US	Partial EMR	39
11.	University of Southern California, Los Angeles, US	Partial EMR	11
12.	University of Pittsburgh Medical Centre, US	Complete EMR	128
13.	University Health Network, Canada	Partial EMR	228
Transplant centre in Australia			
14.	The Royal Adelaide Hospital, Adelaide, Australia	Complete EMR	30

Table 3. Patient demographic characteristics, hospitalization details, co-morbid conditions, immunosuppressive agents used by voriconazole exposure (≥ 1 day exposure to voriconazole *vs.* no exposure to voriconazole) (n=900)

Characteristic	**Voriconazole unexposed** (no exposure to voriconazole) (n =428)		**Voriconazole exposed** (≥ 1 day exposure to voriconazole) (n = 472)		**All Study Patients** (n = 900)	
	#	%	#	%	#	%
Demographic characteristics						
Age (years)						
18-29	48	11.2	97	20.6	145	16.1
30-49	119	27.8	132	28	251	27.9
50-59	163	38.1	110	23.3	273	30.3
60-69	97	22.7	116	24.6	213	23.7
>70	1	0.2	17	3.6	18	2.0
Gender						
Male	218	50.9	260	55.1	478	53.1
Female	210	49.1	212	44.9	422	46.9
Race/Ethnicity						
Caucasian	182	42.5	196	41.5	378	42
Hispanic	7	1.6	6	1.3	13	1.4
African-American	2	0.5	4	0.8	6	0.7
Asian	1	0.2	1	0.2	2	0.2
Other	2	0.5	2	0.4	4	0.4
Missing	234	54.7	263	55.7	497	55.2
Occupation [a]						
Indoor	130	30.4	255	54	385	42.8
Outdoor	21	4.9	36	7.6	57	6.3
Mixed	277	64.7	181	38.3	458	50.9
Chemical exposure [b]						
No	396	92.5	428	90.7	824	91.6
Yes	32	7.5	44	9.3	76	8.4
Geographical Location						
Australia	15	3.5	15	3.2	30	3.3
Canada	149	34.8	79	16.7	228	25.3
France	4	0.9	23	4.9	27	3.0
Germany	94	22	101	21.4	195	21.7
Italy	26	6.1	0	0.0	26	2.9
Netherlands	64	15	27	5.7	91	10.1
Spain	59	13.8	25	5.3	84	9.3
Switzerland	10	2.3	7	1.5	17	1.9
United States	7	1.6	195	41.3	202	22.4
Sun exposure [c]						
Low	172	40.2	159	33.7	331	36.8
Medium	235	54.9	280	59.3	515	57.2
High	21	4.9	33	7.0	54	6.0

Characteristic	Voriconazole unexposed (no exposure to voriconazole) (n =428)		Voriconazole exposed (≥ 1 day exposure to voriconazole) (n = 472)		All Study Patients (n = 900)	
	#	%	#	%	#	%
Clinical characteristics						
Lung transplant type						
Double	332	77.6	379	80.3	711	79
Heart/Lung	14	3.3	13	2.8	27	3.0
Left Single	38	8.9	41	8.7	79	8.8
Right Single	44	10.3	39	8.3	83	9.2
Re-Lung transplant						
No	409	95.6	449	95.1	858	95.3
Yes	19	4.4	23	4.9	42	4.7
Underlying Disease						
Alpha-1 antitrypsin	29	6.8	18	3.8	47	5.2
Cystic fibrosis	69	16.1	132	28	201	22.3
Chronic obstructive pulmonary disease	140	32.7	118	25	258	28.7
Interstitial pulmonary fibrosis	99	23.1	121	25.6	220	24.4
Bronchiolitis obliterans	11	2.6	8	1.7	19	2.1
Primary pulmonary hypertension	14	3.3	14	3.0	28	3.1
Sarcoidosis	7	1.6	9	1.9	16	1.8
Scleroderma	9	2.1	10	2.1	19	2.1
Interstitial Lung Disease	6	1.4	3	0.6	9	1.0
Other	44	10.3	39	8.3	83	9.2
Immune disorder [d]						
No	420	98.1	465	98.5	885	98.3
Yes	8	1.9	7	1.5	15	1.7
SCC prior to LT						
No	428	100	472	100	900	100
Yes	0	0	0	0	0	0
Other cancer pre-LT						
Yes	2	0.5	0	0.0	2	0.2
No	426	99.5	472	100	898	99.8
Dialysis 30 days post LT						
No	414	96.7	459	97.2	873	97
Yes	14	3.3	13	2.8	27	3.0
Transplant rejection episodes						

Characteristic	Voriconazole unexposed (no exposure to voriconazole) (n =428)		Voriconazole exposed (≥ 1 day exposure to voriconazole) (n = 472)		All Study Patients (n = 900)	
	#	%	#	%	#	%
0	240	56.1	213	45.1	453	50.3
1-2	136	31.8	184	39	320	35.6
3-4	41	9.6	46	9.7	87	9.7
>4	11	2.6	29	6.1	40	4.4
Neutropenia episodes[f]						
0	183	42.8	157	33.3	340	37.8
1-2	140	32.7	152	32.2	292	32.4
3-4	63	14.7	63	13.3	126	14
>4	42	9.8	100	21.2	142	15.8
Diabetes post-LT						
No	332	77.6	328	69.5	660	73.3
Yes	83	19.4	122	25.8	205	22.8
Missing	13	3.0	22	4.7	35	3.9
CMV						
D- R-	84	19.6	105	22.2	189	21.0
D+ R+	112	26.2	142	30.1	254	28.2
D- R+	102	23.8	86	18.2	188	20.9
D+ R-	67	15.7	101	21.4	168	18.7
Missing	63	14.7	38	8.1	101	11.2
Days in hospital at the time of LT						
1-14	62	14.5	71	15.0	133	14.8
15-30	151	35.3	191	40.5	342	38.0
>30	157	36.7	153	32.4	310	34.4
Missing	58	13.6	57	12.1	115	12.8
Days in ICU at the time of LT						
1-14	34	7.9	148	31.4	182	20.2
15-30	19	4.4	62	13.1	81	9.0
>30	41	9.6	105	22.2	146	16.2
Missing	334	78.0	157	33.3	491	54.6
Immunosuppressive agents						
Basiliximab						
No	227	53	288	61	515	57.2
Yes	192	44.9	105	22.2	297	33
Missing	9	2.1	79	16.1	88	9.8
Daclizumab						
No	417	97.4	387	82	804	89.3
Yes	2	0.5	4	0.8	6	0.7
Missing	9	2.1	81	17.2	90	10

Characteristic	Voriconazole unexposed (no exposure to voriconazole) (n =428)		Voriconazole exposed (≥ 1 day exposure to voriconazole) (n = 472)		All Study Patients (n = 900)	
	#	%	#	%	#	%
Alemtuzumab						
No	419	97.9	267	56.6	686	76.2
Yes	0	0.0	126	26.7	126	14
Missing	9	2.1	79	16.7	88	9.8
Anti-thymocyte globulin						
No	407	95.1	366	77.5	773	85.9
Yes	10	2.3	27	5.7	37	4.1
Missing	11	2.6	79	16.7	90	10
Supratherapeutic CNI episodes [g]						
0	195	45.6	206	43.6	401	44.6
1-2	162	37.9	190	40.3	352	39.1
3-4	43	10	54	11.4	97	10.8
>4	28	6.5	22	4.7	50	5.6
Immunosuppression [1]						
Cyclosporine use						
No	149	34.8	233	49.4	382	42.4
Yes	279	65.2	239	50.6	518	57.6
Tacrolimus use						
No	173	40.4	91	19.3	264	29.3
Yes	255	59.6	381	80.7	636	70.7
Steroid use						
No	11	2.6	1	0.2	12	1.3
Yes	417	97.4	471	99.8	888	98.7
Mycophenolate use						
No	139	32.5	65	13.8	204	22.7
Yes	289	67.5	407	86.2	696	77.3
Azathioprine use						
No	228	53.3	335	71	563	62.6
Yes	200	46.7	137	29	337	37.4
Sirolimus use						
No	413	96.5	421	89.2	834	92.7
Yes	15	3.5	51	10.8	66	7.3
Everolimus use						
No	391	91.4	442	93.6	833	92.6
Yes	37	8.6	30	6.4	67	7.4

Characteristic	Voriconazole unexposed (no exposure to voriconazole) (n =428)		Voriconazole exposed (≥ 1 day exposure to voriconazole) (n = 472)		All Study Patients (n = 900)	
	#	%	#	%	#	%
Immune score [j]						
≤ 4	30	7.0	38	8.1	68	7.6
>4 and ≤ 6	91	21.3	122	25.8	213	23.7
>6 and ≤ 8	126	29.4	142	30.1	268	29.8
>8 and ≤ 10	79	18.5	92	19.5	171	19.0
>10	102	23.8	78	16.5	180	20.0
Potentially phototoxic drug exposure						
Phototoxic drug use [h]						
No	327	76.4	345	73.1	672	74.7
Yes	101	23.6	127	26.9	228	25.3

[a] Subjectively classified according to whether subject would spend majority of time indoors/outdoors/mixed

[b] Includes insecticides/herbicides/fungicides, petroleum/diesel/tar products, dry cleaning agents, asbestos, fiberglass

[c] According to respective study centre's geographical location by latitude: Low (>45° Latitude), Medium (35-45° Latitude), High (<35° Latitude)

[d] Includes rheumatoid arthritis, systemic lupus erythematosus, Henoch-Schonlein purpura, psoriasis

[e] Not including SCC, BCC, and melanoma

[f] Absolute neutrophil counts < 500 cells/mm^3

[g] Elevated CNI levels were defined as cyclosporine trough >350 mcg/L or tacrolimus trough >20 mcg/L

[h] Includes doxycycline, hydroxychloroquine, nifedipine, diltiazem, glyburide, naproxen, piroxicam, isotretinoin

[i] Patients receiving at least one dose were classified as being exposed

[j] Score based on milligram dose of each maintenance immunosuppressant medication. One unit of immunosuppression was assigned to the corresponding daily doses as follows:

Medication	Unit dose mg/day
Cyclosporine	100
Tacrolimus	2
Prednisone	5
Azathioprine	100
Mycophenolate sodium	360
Mycophenolate mofetil	500
Sirolimus	2
Everolimus	1.5

Table 4. Incidence rate (per 1,000 person-years) of SCC of the skin by four treatment exposure categories: overall, by time since LT, and by patient demographic and clinical characteristics (n=900)

Characteristic	Unexposed	Exposure to voriconazole alone	Exposure to other azole alone	Exposure to voriconazole and other azole(s)
	Per 1,000 person-years	Per 1,000 person-years	Per 1,000 person-years	Per 1,000 person-years
Overall incidence rate				
In all patients (# of SCC cases/person-years)	**13.1** (17/1,299)	**33.4** (28/837)	**10.4** (5/481)	**21.7** (5/230)
Incidence rate by time since LT				
Year-1 post LT (# of SCC cases/person-years)	2.4 1/415	4.4 1/227	0 0/118	34.2 1/29
Year-2 post LT (# of SCC cases/person-years)	4.3 3/698	20.3 9/444	4.2 1/238	10.4 1/96
Year-3 post LT (# of SCC cases/person-years)	7.5 7/934	17.8 11/618	8.9 3/336	12.6 2/158
Year-4 post LT (# of SCC cases/person-years)	8.9 10/1126	28.1 21/749	9.7 4/413	24.6 5/203
Year-5 post LT (# of SCC cases/person-years)	9.1 11/1209	33.7 27/802	8.9 4/447	22.9 5/219
Year-6 post LT (# of SCC cases/person-years)	11.1 14/1266	34.0 28/824	10.7 5/468	22.1 5/226
Year-7 post LT (# of SCC cases/person-years)	13.1 17/1294	33.6 28/834	10.5 5/478	21.7 5/230
Incidence rate across demographic characteristics				
Age (years) 18-29 30-49 50-59 ≥60	 0 0 21.5 21.7	 5.6 46.6 27.7 48.2	 0 0 10.1 34.5	 0 22.5 47.8 30.6

Characteristic	Unexposed	Exposure to voriconazole alone	Exposure to other azole alone	Exposure to voriconazole and other azole(s)
	Per 1,000 person-years	Per 1,000 person-years	Per 1,000 person-years	Per 1,000 person-years
Gender				
Male	16.9	37.5	16.2	8.5
Female	8.5	28.0	6.8	35.3
Race				
Other	0	37.0	0	0
Caucasian	10.7	42.9	7.8	26.5
Missing	19.1	18.9	13.4	16.2
Occupation[a]				
Indoors	6.1	39.0	12.8	39.5
Outdoors	44.8	48.8	29.9	0
Indoors/outdoors	13.3	20.8	4.7	0
Chemical[b]	0	37.8	0	0
Country				
Australia	49.2	168.1	0	0
Canada	17.9	8.2	22.8	0
France	0	0	0	0
Germany	0	0	7.1	0
Italy	0	No patients	0	No patients
The Netherlands	16.6	0	0	0
Spain	0	16.4	0	0
Switzerland	0	0	No patients	0
United States	0	45.3	0	185.4
Sun exposure[c]				
Low	12.1	0	6.3	0
Medium	10.2	33.4	20.5	98.0
High	44.4	103.8	0	0
Incidence rate across clinical characteristics and immunosuppressive agents				
LT type				
Double	13.9	27.8	12.4	24.3
Heart-Lung	0	143.4	0	0
Single (Left/Right)	12.4	54.6	0	0
Lung re-transplant				
No	15.9	37.3	8.2	23.8
Yes	0	0	0	36.6

Characteristic	Unexposed	Exposure to voriconazole alone	Exposure to other azole alone	Exposure to voriconazole and other azole(s)
	Per 1,000 person-years	Per 1,000 person-years	Per 1,000 person-years	Per 1,000 person-years
Underlying disease				
CF	0	11.2	0	9.7
COPD	17.5	21.8	18.9	0
AT	26.5	0	20.0	0
IPF	22.3	56.4	9.8	68.1
PPH	0	158.3	0	0
Scleroderma	0	170.8	0	0
Other	5.9	30.4	0	56.2
Immune disorder[d] prior to LT				
No	12.6	33.9	10.5	21.9
Yes	39.9	0	0	0
Other cancer[e]				
No	12.4	33.4	8.4	21.7
Yes	31.9	NA	349.8	0
Duration of hospital stay (days)				
1-14	19.4	52.1	13.7	197.3
15-30	12.6	24.6	9.1	20.9
>30	11.6	24.5	14.1	0
Duration in ICU (days)				
1-5	20.9	38.9	0	105.3
6-10	11.2	29.6	0	0
>10	6.4	16.3	33.6	0
Number of rejection episodes during follow up				
0	14.7	37.0	3.7	0
1-2	13.3	39.8	29.1	48.8
3-4	11.6	20.9	0	60.8
>4	0	0	0	0
Number of neutropenia episodes during follow up[f]				
0	10.9	52.0	5.8	9.9
1-2	16.2	25.8	5.6	27.5
3-4	13.2	16.8	11.7	0
>4	10.9	31.2	46.9	57.9

Characteristic	Unexposed	Exposure to voriconazole alone	Exposure to other azole alone	Exposure to voriconazole and other azole(s)
	Per 1,000 person-years	Per 1,000 person-years	Per 1,000 person-years	Per 1,000 person-years
Diagnosed with diabetes anytime post transplant				
No	14.3	39.9	13.3	19.8
Yes	10.5	19.5	0	27.8
Required dialysis within 30 days of LT				
No	13.3	33.5	10.8	22.7
Yes	0	0	0	0
CMV				
D-R-	23.7	27.7	12.4	0
D+R+	3.2	31.4	6.6	19.0
D-R+	14.2	37.9	16.4	54.5
D+R-	21.0	28.9	9.9	28.9
Basiliximab(Simulect)				
No	15.8	35.6	12.2	38.2
Yes	11.0	30.2	11.1	38.6
Daclizumab(Zenapax)				
No	13.4	34.5	12.3	31.8
Yes	0	0	0	202.9
Alemtuzumab(Campath)				
No	13.8	32.8	12.0	32.0
Yes	0	36.9	0	177.0
Anti-thymocyte globulin				
No	13.7	37.8	12.2	42.7
Yes	0	0	0	0
Cyclosporine				
No	7.3	36.4	0	85.4
Yes	17.4	27.8	12.9	0
Tacrolimus				
No	16.9	27.0	17.1	0
Yes	10.9	34.4	4.1	40.2

Characteristic	Unexposed	Exposure to voriconazole alone	Exposure to other azole alone	Exposure to voriconazole and other azole(s)
	Per 1,000 person-years	**Per 1,000 person-years**	**Per 1,000 person-years**	**Per 1,000 person-years**
Prednisone/methylprednisolone No Yes	 0 13.3	 No patients 33.4	 No patients 10.4	 No patients 21.7
Mycophenolate No Yes	 21.3 5.8	 32.5 33.7	 34.3 5.1	 0 23.2
Azathioprine No Yes	 1.7 22.6	 33.1 34.2	 6.4 17.8	 25.7 0
Sirolimus/Rapamycin No Yes	 13.6 0	 32.0 52.5	 10.7 0	 14.2 105.7
Everolimus No Yes	 13.5 0	 34.8 0	 12.8 0	 27.2 0
Combined immunosuppressive agents[g]				
Cyclosporine/Mycophenolate	0	0	14.5	0
Cyclosporine/Azathioprine	31.4	47.5	96.8	0
Tacrolimus/Mycophenolate	0	36.0	0	95.4
Tacrolimus/Azathioprine	31.2	0	29.1	0
Rapamycin	0	63.9	0	10.8
Other	10.6	33.0	0	0

[a] Subjectively classified according to whether subject would spend majority of time indoors/outdoors/mixed

[b] Includes insecticides/herbicides/fungicides, petroleum/diesel/tar products, dry cleaning agents, asbestos, fibreglass

[c] According to respective study centre's geographical location by latitude: Low (>45° Latitude), Medium (35-45° Latitude), High (<35° Latitude)

[d] Includes rheumatoid arthritis, systemic lupus erythematosus, Henoch-Schonlein purpura, psoriasis

[e] Not including SCC, BCC, and melanoma

[f] Absolute neutrophil counts < 500 cells/mm^3

[g]. Combined immunosuppressive agents are usually prescribed

Note. Exposure to voriconazole and other azoles were analysed as time-varying covariates.

Table 5. Univariate analyses evaluating the association between four treatment exposure categories and the risk of SCC of the skin in patients with lung or lung/heart transplant (n=900)

Characteristics	**Hazard Ratio**	**95% CI**		**P-value**
		Lower	**Upper**	
Treatment exposure categories				
Unexposed	Ref			
Exposure to voriconazole alone	2.55	1.42	4.60	0.002
Exposure to other azole alone	0.73	0.27	1.98	0.541
Exposure to voriconazole and other azole(s)	1.47	0.53	4.05	0.455
Age (years)				
18-29	Ref			
30-49	6.66	0.88	50.70	0.067
50-59	9.22	1.25	68.20	0.030
≥60	15.04	2.05	110.08	0.008
Gender				
Female	Ref			
Male	1.49	0.86	2.56	0.153
Race/Ethnicity				
Other	Ref			
Caucasian	2.08	0.30	14.52	0.459
Missing	1.51	0.22	10.50	0.674
Occupation [a]				
Indoor	Ref			
Outdoor	1.57	0.69	3.56	0.281
Mixed	0.46	0.26	0.83	0.009
Chemical exposure [b]				
No	Ref			
Yes	0.69	0.21	2.25	0.537
Geographical Location				
Spain	Ref			
Australia	24.8	3.17	193.21	0.002
Canada	5.73	0.75	43.65	0.092
France	-	-	-	-
Germany	1.68	0.15	18.41	0.673
Italy	-	-	-	-
Netherlands	4.56	0.51	40.6	0.174
Switzerland	-	-	-	-
United States	19.5	2.63	144.84	0.004
Sun exposure [c]				
Low	Ref			
Medium	3.37	1.42	8.0	0.006
High	4.40	3.50	23.49	<0.001

Characteristics	Hazard Ratio	95% CI		P-value
		Lower	Upper	
Lung transplant type				
Heart/Lung	Ref			
Double	1.44	0.19	10.82	0.724
Single (right or left)	1.80	0.22	14.41	0.581
Lung re-transplant				
No	Ref			
Yes	0.41	0.59	2.84	0.366
Underlying Disease				
Cystic fibrosis	Ref			
Chronic obstructive pulmonary disease	3.08	1.04	9.10	0.041
Alpha-1 antitrypsin	3.37	0.76	14.99	0.111
Interstitial pulmonary fibrosis	6.20	2.15	17.91	0.001
Primary pulmonary hypertension	4.82	1.08	21.55	0.039
Scleroderma	5.69	1.00	32.44	0.050
Other	2.97	0.80	11.08	0.105
Immune disorder [d]				
No	Ref			
Yes	1.13	0.19	6.83	0.897
Other cancer pre-LT [e]				
No	Ref			
Yes	22.06	9.97	48.81	<0.001
Dialysis 30 days post LT				
No	Ref			
Yes	-	-	-	-
Transplant rejection episodes				
0	Ref			
1-2	1.59	0.91	2.79	0.107
3-4	0.84	0.32	2.22	0.722
>4	-	-	-	-
Neutropenia episodes[f]				
0	Ref			
1-2	0.92	0.48	1.77	0.800
3-4	0.66	0.26	1.65	0.371
>4	1.39	0.69	2.78	0.354
Diabetes post-LT				
No	Ref			
Yes	0.66	0.32	1.36	0.26
CMV				
D- R-	Ref			
D+ R+	0.73	0.33	1.60	0.431

Characteristics	Hazard Ratio	95% CI		P-value
		Lower	Upper	
D- R+	1.10	0.52	2.34	0.805
D+ R-	1.11	0.52	2.41	0.785
Days in hospital at the time of LT				
1-14	Ref			
15-30	0.48	0.24	0.94	0.033
>30	0.42	0.20	0.87	0.020
Days in ICU at the time of LT				
1-14	Ref			
15-30	0.56	0.21	1.51	0.252
>30	0.40	0.17	0.98	0.045
IL-2 antagonist				
No	Ref			
Yes	0.67	0.38	1.20	0.176
Alemtuzumab				
No	Ref			
Yes	2.44	1.23	4.80	0.010
Antithymocyte globulin use				
No	Ref			
Yes	-	-	-	-
Supratherapeutic CNI episodes [g]				
0	Ref			
1-2	0.69	0.37	1.30	0.253
3-4	1.30	0.64	2.66	0.463
>4	1.18	0.42	3.32	0.752
Cyclosporine use				
No	Ref			
Yes	0.64	0.38	1.08	0.094
Tacrolimus				
No	Ref			
Yes	1.04	0.58	1.86	0.898
Steroid use				
No	Ref			
Yes	-	-	-	-
Mycophenolate				
No	Ref			
Yes	0.60	0.35	1.04	0.067
Azathioprine				
No	Ref			
Yes	1.21	0.71	2.07	0.49

Characteristics	Hazard Ratio	95% CI		P-value
		Lower	Upper	
Sirolimus				
No	Ref			
Yes	1.37	0.53	3.57	0.518
Everolimus				
No	Ref			
Yes	-	-	-	-
Immunosuppression Regimen [h]				
Cyclosporine/Mycophenolate	Ref			
Cyclosporine/Azathioprine	7.11	1.56	32.50	0.011
Tacrolimus/Mycophenolate	4.35	1.00	18.99	0.05
Tacrolimus/Azathioprine	4.51	0.88	23.04	0.07
Rapamycin	4.32	0.81	23.07	0.086
Other	1.69	0.39	7.44	0.485
Phototoxic drug [i]				
No	Ref			
Yes	0.68	0.35	1.31	0.247

[a] Subjectively classified according to whether subject would spend majority of time indoors/outdoors/mixed

[b] Includes insecticides/herbicides/fungicides, petroleum/diesel/tar products, dry cleaning agents, asbestos, fibreglass

[c] According to respective study centre's geographical location by latitude: Low (>45° Latitude), Medium (35-45° Latitude), High (<35° Latitude)

[d] Includes rheumatoid arthritis, systemic lupus erythematosus, Henoch-Schonlein purpura, psoriasis

[e] Not including SCC, BCC, and melanoma

[f] Absolute neutrophil counts < 500 cells/mm^3

[g] Elevated CNI levels were defined as cyclosporine trough >350 mcg/L or tacrolimus trough >20 mcg/L

[h] Combined immunosuppressive agents are usually prescribed. Calcineurin inhibitors, including cyclosporine and tacrolimus have been the cornerstones of an immunosuppressive regimen, which usually includes 2 or more additional agents, almost always glucocorticoids, and a purine antagonist (mycophenolic acid or azathioprine). Sirolimus (rapamycin) has been used as a substitute for CNIs. The choice of agents is often immunosuppressive- protocol driven but is usually adapted to each recipient's risk profile or intolerance to one of these agents.

[i.] Includes doxycycline, hydroxychloroquine, nifedipine, diltiazem, glyburide, naproxen, piroxicam, isotretinoin

Note. Exposure to voriconazole, other azole and immunosuppressive agents were analysed as time-varying covariate.

Table 6. Multivariable analyses to evaluate the association between four treatment exposure categories and the risk of SCC of the skin in patients with lung or lung/heart transplant controlling for the effect of potential confounders (n=900)

Characteristic	Hazard Ratio	95% Confidence Interval		P-value
Treatment exposure categories[a]				
Unexposed	Ref	-	-	-
Exposure to voriconazole alone	2.39	1.31	4.37	0.005
Exposure to other azole alone	0.80	0.26	2.49	0.698
Exposure to voriconazole and other azole(s).	3.45	1.07	11.06	0.038
Immunosuppression Regimen[b]				
Cyclosporine/mycophenolate	Ref	-	-	-
Cyclosporine/azathioprine	6.48	1.33	31.42	0.020
Tacrolimus/mycophenolate	1.54	0.24	9.92	0.652
Tacrolimus/azathioprine	2.53	0.31	20.44	0.384
Rapamycin	1.89	0.28	12.80	0.513
Other	1.16	0.21	6.50	0.864
Mean Cyclosporine level (20 mcg/L increment) [c]	1.02	0.97	1.07	0.501
Mean tacrolimus level (1 mcg/L increment) [c]	1.09	1.02	1.16	0.006
Transplant rejection episodes				
0	Ref			
≥1	1.18	0.62	2.24	0.614
Sun exposure[d]				
Low	Ref	-	-	-
Medium	1.60	0.53	4.81	0.402
High	6.67	2.29	19.41	0.001
Gender				
Female	Ref	-	-	-
Male	1.43	0.80	2.57	0.229
Age (years)				
18-29	Ref	-	-	-
30-49	5.16	0.68	39.06	0.112
50-59	7.10	0.83	60.60	0.073
≥ 60	8.66	1.02	73.33	0.048

Characteristic	Hazard Ratio	95% Confidence Interval		P-value
History of malignancy pre-LT				
No	Ref	-	-	-
Yes	18.99	4.82	74.85	<0.001
Underlying diseases				
Cystic fibrosis	Ref	-	-	-
Chronic obstructive pulmonary disease	1.89	0.57	6.30	0.301
Alpha-1 antitrypsin deficiency	3.77	0.85	16.77	0.081
Interstitial pulmonary fibrosis	3.98	1.25	12.72	0.020
Primary pulmonary hypertension	5.08	1.16	22.17	0.031
Scleroderma	9.58	1.56	58.79	0.015
Other	3.02	0.82	11.16	0.098

[a] Exposure to voriconazole and other azole was analysed as time-varying covariates.

[b]Immunosuppression: Patients receiving 30-days or more of a specific drug were classified as being exposed to that drug. Analysed as time-varying covariate. Further, Combined immunosuppressive agents are usually prescribed. Calcineurin inhibitors, including cyclosporine and tacrolimus have been the cornerstones of an immunosuppressive regimen, which usually includes 2 or more additional agents, almost always glucocorticoids, and a purine antagonist (mycophenolic acid or azathioprine). Sirolimus (rapamycin) has been used as a substitute for CNIs. The choice of agents is often immunosuppressive- protocol driven but is usually adapted to each recipient's risk profile or intolerance to one of these agents.

[c]Mean calcineurin inhibitor levels: Treated as time-varying covariate.

[d]Sun exposure: According to respective study centre’s geographical location by latitude: Low (>45° Latitude), Medium (35-45° Latitude), High (<35° Latitude).

[e]History of Malignancy: Not including SCC, BCC, and melanoma.

Note: Covariates in the multivariable model included: age, gender, immunosuppression regimen, mean cyclosporine level, mean tacrolimus level, sun exposure, history of malignancy pre-transplant, transplant rejection episodes, underlying disease. HR estimates for treatment exposure categories and other covariates were controlled for all the variables included in the model.

Table 7. Multivariable analyses to evaluate the effect of cumulative duration of voriconazole and other azole on the risk of SCC of the skin in patients with lung or lung/heart transplant controlling for the effect of potential confounders (n=900)

	Hazard Ratio	95% Confidence Interval		P-value
Duration of treatment				
No exposure to any azole	Ref			
Exposure to voriconazole 1-90 days	0.45	0.10	2.10	0.311
Exposure to voriconazole 91-180 days	2.23	0.94	5.30	0.070
Exposure to voriconazole >180 days	3.52	1.59	7.79	0.002
Exposure to other azole 1-90 days	- (no event)	-	-	-
Exposure to other azole 91-180 days	1.59	0.35	7.34	0.551
Exposure to other azole >180 days	1.12	0.24	5.30	0.887

[a] Duration of exposure to voriconazole and other azoles were analysed as time-dependent variables

Note: Covariates in the multivariable model included: age, gender, immunosuppression regimen, mean cyclosporine level, mean tacrolimus level, sun exposure, history of malignancy pre-transplant, transplant rejection episodes, underlying disease. HR estimates for voriconazole and other azole were controlled for all the variables included in the model.

Table 8. Multivariable analyses to evaluate the effect of dose of voriconazole as measured in daily defined dose (DDD) on the risk of SCC of the skin in patients with lung or lung/heart transplant controlling for the effect of potential confounders (n=900)

	Hazard Ratio	95% Confidence Interval		P-value
Mean voriconazole daily dose (per 1 DDD increment)	2.70	1.53	4.78	0.001

[a] Exposure to voriconazole was analysed as a time- dependent variables

Note: Covariates in the multivariable model included: age, gender, immunosuppression regimen, mean cyclosporine level, mean tacrolimus level, sun exposure, history of malignancy pre-transplant, transplant rejection episodes, underlying disease. HR estimates for voriconazole was controlled for all the variables included in the model.

Table 9. Multivariable analyses to evaluate the association between ≥ 1 day voriconazole and the risk of SCC of the skin in lung or lung/heart transplant patients controlling for the effect of potential confounders (n=900)

Treatment Exposure Category[a]	Hazard Ratio	95 % Confidence Interval		P-value
Treatment exposure categories				
No exposure to any azole	Ref			
≥1 day exposure to voriconazole	1.83	0.94	3.55	0.073
≥1 day exposure to other azole	0.59	0.18	1.94	0.384
≥1 day exposure to voriconazole and other azole each	2.15	0.76	6.07	0.147

[a]Voriconazole and other azoles were analysed as time- dependent variables

Note: Covariates in the multivariable model included: age, gender, immunosuppression regimen, mean cyclosporine level, mean tacrolimus level, sun exposure, history of malignancy pre-transplant, transplant rejection episodes, underlying disease. HR estimates for voriconazole and other azole were controlled for all the variables included in the model.

Table 10. Multivariable analyses to evaluate voriconazole exposure ≥ 30 days exposure to voriconazole (*vs.* <30 days of exposure to voriconazole) on the risk of SCC of the skin in patients with lung or lung/heart transplant controlling for the effect of potential confounders (n=900)

Treatment Exposure Category	Hazard Ratio	95% Confidence Interval		P-value
<30 days exposure to voriconazole	Ref	-	-	
≥ 30 days exposure to voriconazole	2.69	1.59	4.55	<0.001

[a]Voriconazole exposure was analysed as a time- dependent variable

Note: Covariates in the multivariable model included: age, gender, immunosuppression regimen, mean cyclosporine level, mean tacrolimus level, sun exposure, history of malignancy pre-transplant, transplant rejection episodes, underlying disease. HR estimates for voriconazole was controlled for all the variables included in the model.

Table 11. Multivariable analyses to evaluate voriconazole exposure ≥ 1 day exposure to voriconazole (*vs*. no exposure to voriconazole) on the risk of SCC of the skin in patients with lung or lung/heart transplant controlling for the effect of potential confounders (n=900)

Treatment Exposure Category	Hazard Ratio	95% Confidence Interval		P-value
No exposure to voriconazole	Ref	-	-	
≥1 day exposure to voriconazole	2.21	1.31	3.72	0.003

[a]Voriconazole was analysed as a time- dependent variable

Note: Covariates in the multivariable model included: age, gender, immunosuppression regimen, mean cyclosporine level, mean tacrolimus level, sun exposure, history of malignancy pre-transplant, transplant rejection episodes, underlying disease. HR estimates for voriconazole was controlled for all the variables included in the model.

Appendix- I

Considerations for time-varying exposure analysis in PASS A1501097

Steps for defining start of "at risk period", relative to lung transplant, of clinically meaningful exposure (≥ 30 days) to an anti-fungal (AF) therapy

1. Let M = minimal cumulative number of days exposed to the AF considered to be "clinically meaningful exposure"; these days need not be consecutive days.
2. If the total number of days exposed to the AF is < M, assume no significant exposure and treat as unexposed to that AF. For example, if M=30 and the patient took the AF on days 5-19 and day 11, then the total number of days exposed is 20, and therefore the patient did not have clinically meaningful exposure.

3. If the total number of days exposed to the AF is >= M, set the M^{th} day of cumulative exposure to the AF as the start day for clinically meaningful exposure to that AF. For example, if M=30 and the patient was exposed to the AF on days 5-20 and days 25-100, then the total number of days exposed is 92, and the start day of significant exposure to AF is day 39^{th} ; i.e., the 30^{th} day at which they took the AF.

Time-dependent covariate definition

After determining the start day of significant exposure for each AF, including voriconazole, the time-varying exposure value at each time point post-transplant can be determined as follows. Let Z(*t*) denote the value of the time-varying exposure covariate at time t. For each patient, Z(*t*) is defined from lung transplantation (t=0) on up to the earliest of: first occurrence of the event (SCC), or end of patient follow-up. At each time point it takes the values of 0 (previously not exposed to any AF), 1 (previously exposed to voriconazole, but not another AF), 2 (previously exposed to another AF but not exposed to voriconazole), or 3 (if previously exposed to voriconazole and another AF), as detailed below:

Z(*t*) = 0, if there was no significant exposure to any AF that started on or before day *t*. I.e., all clinically meaningful exposures to AF (if any) started after *t*.

= 1, if significant exposure to voriconazole started on or before day *t* and all significant exposures to other AFs
(if any) started after *t*.
= 2, if significant exposure to any other AF started on or before day *t* and significant exposure to voriconazole
(if any) started after *t*.
= 3, if significant exposure to voriconazole started on or before day *t* and significant exposure to another AF
started on or before day *t*

Note that while the start day of significant exposure of voriconazole and/or other AF is taken into account, the last day of exposure does not enter into the definition above; this means that the effect modelled for significant exposure has an “onset” time (start day), but it does not have an “offset” time. Also, the definition combines all non- voriconazole AFs in one group.