Title

Voriconazole Exposure and Risk of Cutaneous Squamous Cell Carcinoma, Aspergillus Colonization, Invasive Aspergillosis and Death in Lung Transplant Recipients

Journal

American Journal of Transplantation, 16(1)

ISSN

1600-6135

Authors

Mansh, M

Binstock, M

Williams, K

et al.

Publication Date

2016

DOI

10.1111/ajt.13431

Peer reviewed

HHS Public Access

Author manuscript

Am J Transplant. Author manuscript; available in PMC 2017 January 01.

Published in final edited form as:

Am J Transplant. 2016 January ; 16(1): 262–270. doi:10.1111/ajt.13431.

Voriconazole Exposure and Risk of Cutaneous Squamous Cell Carcinoma, *Aspergillus* Colonization, Invasive Aspergillosis and Death in Lung Transplant Recipients

M. Mansh[1,2], **M. Binstock**[1], **K. Williams**[1], **F. Hafeez**[1], **J. Kim**[1], **D. Glidden**[3], **R. Boettger**[4], **S. Hays**[5], **J. Kukreja**[6], **J. Golden**[5], **M.M. Asgari**[7], **P. Chin-Hong**[5], **J.P. Singer**[5], and **S. Arron**[1]

[1]Department of Dermatology, University of California, San Francisco, California

[2]Stanford University School of Medicine, Stanford, California

[3]Department of Epidemiology and Biostatistics, University of California, San Francisco, California

[4]Department of Clinical Pharmacy, University of California, San Francisco, California

[5]Department of Medicine, University of California, San Francisco, California

[6]Department of Surgery, University of California, San Francisco, California

[7]Division of Research, Kaiser Permanente Northern California, Oakland, California

Abstract

Voriconazole is a triazole antifungal used to prevent and treat invasive fungal infections after lung transplantation, but has been associated with an increased risk for developing cutaneous squamous cell carcinoma (SCC). Despite widespread use, there are no clear guidelines for optimal prophylactic regimens that balance its competing risks and benefits. We conducted a retrospective cohort study of all lung transplant recipients at the University of California, San Francisco transplanted between October 1991 and December 2012 (n=455) to investigate whether voriconazole exposure impacted development of SCC, *Aspergillus* colonization, invasive aspergillosis, and all-cause mortality. Voriconazole exposure was associated with a 73% increased risk for developing SCC (HR=1.73; 95% CI: 1.04–2.88; p=0.03), with each additional 30-day exposure at the standard dose increasing the risk by 3.0% (HR=1.03; 95% CI: 1.02–1.04; p<0.001). Voriconazole exposure reduced risk of *Aspergillus* colonization by 50% (HR=0.50; 95% CI: 0.34–0.72; p<0.001), but we were underpowered to detect risk reduction for invasive aspergillosis. Voriconazole exposure significantly reduced all-cause mortality among subjects who developed *Aspergillus* colonization (HR=0.34; 95% CI: 0.13–0.91; p=0.03), but had no significant

Corresponding author: Sarah Arron, M.D., PhD., UCSF Dermatologic Surgery and Laser Center, 1701 Divisadero St, Third Fl, Box 316, San Francisco, CA 94115, Phone: 415.514.9769, Fax: 415.476.8837, arrons@derm.ucsf.edu.

Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1. Demographic Characteristics, Cases vs. Controls by Primary Outcomes

Table S2. "Ever" Exposure Expanded Multivariate Cox Regression Models by Primary Outcomes

Table S3. Expanded Hazard Ratio (eHR) for Invasive Aspergillosis and All-Cause Mortality, by Voriconazole Exposure and Stratified by *Aspergillus* Colonization Status

impact on those without colonization. Physicians should consider patient-specific factors that modify the potential risks and benefits of voriconazole in the care of lung transplant recipients.

Introduction

Skin cancer is the most common malignancy following solid organ transplantation. Notably, organ transplant recipients experience a more than 65-fold increased risk of developing cutaneous squamous cell carcinoma (SCC) compared to the general population.[1] SCCs that develop in organ transplant recipients behave aggressively and can lead to a large number of cutaneous lesions resulting in multiple debilitating surgeries and increased risk of death.[2,3] Lung transplant recipients are particularly susceptible due to older age at transplant and more intensive immunosuppression.[4–8]

Lung transplant recipients also have high rates of fungal infections after transplantation (15–35%), which can result in significant morbidity and mortality (up to 78% for invasive infections).[9] Many lung transplant programs implement universal antifungal prophylaxis after transplant based on evidence that it may reduce incidence of invasive fungal infections and death.[10] Targeted prophylaxis or treatment is also often reinstituted in patients with evidence of fungal colonization and/or those requiring increased immunosuppression used to treat acute allograft rejection. Voriconazole is a broad-spectrum triazole antifungal first approved in 2002 for the treatment and prevention of invasive fungal infections. In a recent world-wide survey,[11] voriconazole-based antifungal prophylaxis regimens were the most commonly utilized in lung transplant recipients. This is likely due to the drug's relative ease of administration and effectiveness compared to other therapeutics.[12]

Retrospective cohort and case-control studies have demonstrated that voriconazole increases the risk for developing SCC in lung transplant recipients.[13–15] Our group recently showed that any voriconazole exposure is associated with a 2.6 fold increased risk for SCC in lung transplant recipients and that the risk is dose-dependent.[16] While the exact mechanism of carcinogenesis is not well understood, it may result from drug-associated phototoxicity.[17,18]

No clear guidelines exist for the optimal dose and duration of voriconazole prophylaxis in lung transplant recipients. Importantly, prior studies evaluating voriconazole exposure and risk for SCC have not balanced this finding against the drug's potential for reducing invasive fungal infections and mortality. To evaluate the relative risks and benefits of voriconazole in lung transplant recipients, we conducted an expanded 21-year single-center retrospective cohort study.

Materials and Methods

Cohort Population

To investigate the effect of voriconazole exposure on the risk of squamous cell carcinoma (SCC), *Aspergillus* colonization, invasive aspergillosis, and all-cause mortality after lung transplantation, we performed a single-center, retrospective cohort study of all patients who underwent single lung, double lung, or heart-lung transplantation at the University of California at San Francisco (UCSF) from October 23, 1991 to December 27, 2012 (n=455).

This study was approved by the UCSF Committee on Human Research and was performed in compliance with the Declaration of Helsinki.

Measurement of Drug Exposure

At UCSF, all lung transplant recipients receive universal anti-fungal prophylaxis for 3 months following transplantation starting on post-operative day 3. Post-transplant prophylaxis is extended, or therapy reinstituted, at the discretion of the treating physician, for treatment of fungal colonization of the respiratory tract or when immunosuppression is increased as a result of an acute rejection episode. Most invasive infections are treated for 6–8 weeks. Between 1991–2002, inhaled amphotericin was utilized by protocol for both prophylaxis against and treatment for *Aspergillus* colonization and invasive fungal infections. In 2002, voriconazole was first introduced into the care of lung transplant recipients at UCSF for treatment of *Aspergillus* colonization and invasive fungal infections. In 2005, voriconazole replaced inhaled amphotericin as the first-line medication for prophylaxis.

Drug exposure information for outpatient administration of voriconazole, inhaled amphotericin B, and posaconazole was obtained using medical record review as previously described.[16] For the purposes of this study, we standardized post-operative day 3 after lung transplant as our index (start) date for obtaining drug dosing. Patients who underwent re-transplantation (n=14) were assessed from the index date of their first transplant.

Covariates

We assessed risk factors associated with development of SCC, invasive aspergillosis, and death. We acquired all demographic data from the Organ Procurement and Transplantation Network (OPTN) registry (STAR File #020910–16) and medical record review. Covariates include sex, race/ethnicity, age at transplant, Lung Allocation Score (LAS) diagnostic category,[19] type of transplant (single lung, double lung, or heart-lung transplantation), and year of transplant (era effect). Smoking history, a known risk factor for SCC,[20] was missing for 160 (37.9%) patients and was not included. History of CMV disease, a risk factor for invasive aspergillosis in organ transplant recipients,[21] was not evaluated because of low cumulative incidence in our cohort (n=5; 1.1%). We believe this low incidence is related to the fact that all lung transplant recipients at UCSF receive lifelong universal prophylaxis with valganciclovir.[22]

Primary Outcomes

We first screened all medical records for diagnosis of SCC by a pathology or dermatopathology report with an ICD-9 code of 173.x or 232.x. As these codes capture any non-melanoma skin cancer, additional review was used to adjudicate whether these codes referred to SCC based on a line diagnosis of SCC, SCC in situ, Bowen's disease, or keratoacanthoma. Skin cancer outcome data was missing for 7/455 (1.5%) patients and they were excluded from outcome-specific analysis.

Invasive aspergillosis diagnosis was identified by two methods. First, medical records were reviewed for appropriate ICD-9 diagnostic codes (117.3 and 484.6) for invasive

aspergillosis. To ensure the completeness, hospital and laboratory records of all lung transplants were then reviewed to confirm a positive respiratory culture of *Aspergillus*. Potential cases were defined based on established European Organization for Research and Treatment (EORTC) criteria for invasive fungal infection. Individuals meeting the definition of "probable" or "proven" invasive aspergillosis were assigned a positive diagnosis.[6] There were 13/455 (2.9%) patients with missing or insufficient data on invasive aspergillosis and these individuals were excluded from outcome-specific analysis.

We also assessed *Aspergillus* colonization, defined as date of positive culture of any *Aspergillus* species from routine tracheal aspirate or bronchoalveolar lavage in the absence of invasive disease. We acquired dates of death, if applicable, from the OPTN registry.

Statistical Analysis

We used survival analysis to test whether voriconazole exposure impacted development of SCC, *Aspergillus* colonization, invasive aspergillosis, and all-cause mortality. Multivariate Cox proportional hazards regression models were built with modified Allen-Cady backwards selection in order to calculate the relative hazard ratios for each outcome by voriconazole exposure. For each outcome, we developed two, separate analytic models expressing voriconazole drug exposure as either a (i) time-varying covariate of "ever" or "never" voriconazole exposure or as (ii) time-varying covariate of cumulative dose voriconazole exposure as described previously.[16] For the cumulative dose models, a single unit of drug exposure was defined as 12 grams, equivalent to 1 month (30 days) of voriconazole exposure at the standard dose of 200 mg twice daily. In each model, subjects exited the study if they (i) developed the primary outcome (ii) died (iii) were lost to follow-up, or (iv) the study period ended.

Drug exposure data was either incomplete or missing for 68/455 (15.0%) cohort members. The subjects with missing data were similar with respect to age, sex, race, LAS diagnostic category, and type of transplant, but were more likely to have received a transplant in earlier years, less likely to develop squamous cell carcinoma, and more likely to have died by the end of the study period compared to those with complete data. To address this potential bias, we generated inverse weights based on a logistic regression model of missingness on these variables.[23,24]

For multivariate adjustment, sex, race, and age at transplant were kept *a priori* given their known association with SCC.[25] LAS diagnostic category and type of transplant were not significant in any outcome model, but year of transplant was significant in the all-cause mortality outcome model only. Thus, for all outcome analyses, we calculated the relative hazard ratio by drug exposure using an unadjusted Cox regression model (HR), a model adjusted for sex, race, and age at transplant (aHR), and an expanded model that also included year of transplant (eHR). For the SCC outcome only, individuals with a prior history of squamous cell carcinoma before lung transplantation (n=4) were excluded from multivariate analysis by left-censoring. The proportional hazards assumption was tested and confirmed with the Schoenfeld test. The goodness of fit of the models was confirmed by comparing a plot of the Cox-Snell residuals with the Nelson-Aalen cumulative hazard function.

We also conducted additional analysis for both the invasive aspergillosis and all-cause mortality outcome models, stratifying subjects by history of *Aspergillus* colonization status, as we hypothesized that colonization status might modify the relationship between voriconazole exposure and these outcomes.[26] For SCC analysis only, we also developed a cumulative dose exposure model that included voriconazole, inhaled amphotericin B, and posaconazole exposure, to test whether or not alternative medications used for antifungal prophylaxis in lung transplant recipients also impacted development of SCC after lung transplantation (i.e. confounding by indication).

Finally, we used Kaplan-Meier methods to generate unadjusted outcome-free survival plots for our primary outcomes stratifying cohort participants by “ever” or “never” voriconazole exposure. We also calculated the cumulative incidence of our primary outcomes by “ever” or “never” voriconazole exposure at 1, 5, and 10 years post-transplant adjusting for age at transplant, sex, race, and year of transplant. For both analyses, voriconazole exposure status was assessed at the time of study exit (development of primary outcome, death, loss to follow-up, or, end of study period) for each cohort participant in order to capture only exposure that occurred prior to development of the primary outcome.

Statistical analyses were conducted using Stata version 12 (Statacorp, College Station, Texas, USA) with two-sided alpha < 0.05.

Results

Our cohort (n=455) included 98 single-lung, 347 double-lung and 10 heart-lung transplant recipients at UCSF. Individuals were predominately male (53.6%), white race (79.1%), and had a median age at transplant of 55.4 years (range: 14.9–74.4 years) (Table 1). Among subjects with complete data on drug exposure, 327/387 (84.5%) were “ever” exposed to voriconazole and 60/387 (15.5%) were “never” exposed before study exit; “ever” exposure was included in models as a time-varying covariate. The mean duration of voriconazole exposure was 9.8 months (SD: 13.3 months; range: 0–98.7 months). Individuals “ever” exposed to voriconazole were more likely to have undergone lung transplant later during our study period (median year of transplant: 2008, range: 1994–2012) compared to those “never” exposed to voriconazole (median year of transplant: 1998, range 1991–2012).

During the study period, 86/448 (19.20%) patients developed at least one SCC, 119/455 (26.2%) developed post-transplant *Aspergillus* colonization, 76/442 (16.7%) patients developed invasive aspergillosis, and 208/455 (45.7%) patients died (Table S1).

Squamous Cell Carcinoma

Lung transplant recipients who developed SCC (n=86) had a mean duration of 13.2 months of prior voriconazole exposure at SCC diagnosis (SD: 13.9 months, range: 0–67.5 months). “Ever” exposure to voriconazole was associated with a 73% increased risk for developing SCC (aHR=1.73; 95% CI: 1.04–2.88; p=0.03). This relationship was dose-dependent with each additional 12 gram dose of exposure (equivalent to 200 mg twice daily for 30 days) increasing the risk by 3.0% (aHR=1.03; 95% CI: 1.02–1.04; p<0.001). In our expanded model adjusting for year of transplantation, although the effect size remained the same, the

association of “ever” exposure was no longer statistically significant (eHR=1.71; 95% CI: 0.83–3.52; p=0.15). Covariates associated with SCC in previous studies were also associated with SCC in our cohort. These included age ≥ 50 at transplant (eHR=1.87; 95% CI: 1.27–2.75; p=0.001), male sex (eHR=1.67; 95% CI: 1.17–2.38; p=0.005), and white race (eHR=4.25; 95% CI: 1.99–9.04; p<0.001) (Table S2).

In a combined “triple drug” model adjusting for the cumulative dose exposure to voriconazole, inhaled amphotericin B, and posaconazole, we found that only voriconazole (eHR=1.03, 95% CI: 1.02–1.04; p<0.001) and neither inhaled amphotericin B (eHR=1.00, 95% CI: 0.99–1.01; p=0.75) or posaconazole (eHR=0.99, 95% CI: 0.95–1.05; p=0.81) were associated with development of SCC.

Using unadjusted Kaplan Meier methods, “ever” exposure to voriconazole was associated with an absolute risk increase for SCC of 15% at 5 years and 7% at 10 years (Figure 1a). In our cumulative incidence models adjusted for age, sex, race, and year of transplant, we found voriconazole exposed subjects had an absolute risk increase for SCC of 9% at 5 years and 15% at 10 years after transplantation (Table 3).

Aspergillus Colonization and Invasive Aspergillosis

“Ever” exposure to voriconazole was associated with a 50% decreased risk of developing *Aspergillus* colonization after lung transplantation (eHR=0.50; 95% CI: 0.34–0.72; p<0.001), but this relationship was not dose-dependent (Table 2). However, when we restricted analysis to the first year post-transplant, we found an 18% decreased risk of colonization for each 30 days of drug exposure (eHR=0.82; 95% CI: 0.70–0.96; p=0.01). No covariates were significantly associated with risk of colonization (Table S2). In our adjusted cumulative incidence model, we found voriconazole exposure was associated with an absolute risk decrease for colonization of 19% at 5 years and 24% at 10 years post-transplant (Table 3).

Both drug exposure models showed a trend towards drug-related reduction in the risk of develop invasive aspergillosis after transplantation, but neither relationship reached statistical significance (Table 2). Colonization status did not modify this relationship (Table S3) and no covariates were significantly associated with risk of invasive aspergillosis (Table S2). Using unadjusted Kaplan Meier methods, “ever” exposure to voriconazole was associated with an absolute risk decrease for invasive aspergillosis of 5% at both 5 and 10 years (Figure 1d). In our adjusted cumulative incidence models, we found voriconazole exposure was associated with an absolute risk decrease for invasive aspergillosis of 2% at 1 year, 4% at 5 years and 6% at 10 years post-transplant (Table 3).

All-Cause Mortality

“Ever” exposure to voriconazole did not significantly impact all-cause mortality (eHR=1.32; 95% CI: 0.87–1.99; p=0.19) in our overall cohort. Factors that were associated with all-cause mortality included white race (eHR=1.52; 95% CI:1.05–2.21; p=0.03) and year of transplant (eHR=-.91; 95% CI: 0.87–0.95, p<0.001) (Table S2). However, we found that *Aspergillus* colonization status modified the relationship between voriconazole exposure and

mortality in stratified analysis. Voriconazole exposure reduced mortality risk by 66% among subjects colonized with *Aspergillus* (eHR=0.34; 95% CI: 0.13–0.91; p=0.03), but had no significant impact on those without evidence of colonization (eHR=1.49; 95% CI: 0.90–2.48; p=0.12) (Table S3). In our cumulative dose model, we found a 2% increased mortality risk for each 30-day exposure to voriconazole (eHR=1.02, 95% CI: 1.01–1.03; p<0.001) in the overall cohort (Table 2). However, in stratified analysis, we found this increased mortality risk was restricted to subjects without evidence of colonization (eHR=1.02; 95% CI: 1.01–1.04; p<0.001). There was no dose relationship between voriconazole exposure and all-cause mortality among subjects with *Aspergillus* colonization (eHR=1.00; 95% CI: 0.98–1.03; p=0.61) (Table S3).

Using unadjusted Kaplan Meier methods, voriconazole exposure was associated with an absolute risk decrease for all-cause mortality of 14% at 5 years and 8% at 10 years (Figure 1b). However, in the adjusted cumulative incidence model, we found an absolute risk increase for mortality of 9% at 5 years and 10% at 10 years post-transplant in the overall cohort (Table 3). In a sensitivity analysis, we found that the disparity between the results of our unadjusted Kaplan Meier and multivariate adjusted cumulative incidence analyses was primarily due to adjustment for year of transplant.

Discussion

In this retrospective cohort study of 455 lung transplant recipients, we found that voriconazole exposure is associated with an increased risk of developing SCC, but also significantly reduces risk of developing *Aspergillus* colonization after lung transplantation, and, among those that become colonizers, all cause-mortality. We found that voriconazole exposure after lung transplantation is associated with a 73% increased risk of SCC and that this relationship is dose-dependent, with each additional 30-day exposure at the standard dose of 200-mg twice daily increasing the risk by 3%. These results are consistent with our previous cohort study, which demonstrated a 6% increased risk for each 60-day exposure.[16] While the relationship between “ever” exposure to voriconazole and SCC became insignificant after controlling for year of transplant, the stability of the point estimates suggests this was most likely due to a degree of multi-collinearity between year of transplant and “ever” voriconazole exposure. We also extended these previous findings with a larger and more statistically powered study to now demonstrate that inhaled amphotericin-B and posaconazole do not confer an increased risk of SCC in these patients. This finding is important as recent research suggests these medications, including their newer formulations, may be both clinically efficacious[27] and cost-effective[28] for fungal prophylaxis in immunosuppressed patients and potentially represent safer alternatives for subjects with a high risk for SCC. Our findings also contradict the recent suggestion that the association between voriconazole and SCC is a result of confounding by indication.[29] Finally, we validated patient-specific demographic factors that significantly increase SCC risk in lung transplant recipients including male sex, older age at transplant, and white race, which are similar to the general population.[30,31]

Furthermore, for the first time, we also assessed the potential benefits of voriconazole exposure on reducing invasive fungal infections and all-cause mortality in the context of this

risk. We found a statistically insignificant trend that voriconazole exposure reduced invasive aspergillosis, though the low incidence of this outcome in our cohort (n=79/442; 16.47%) limited the power of our study to detect this difference. Voriconazole exposure did provide a 50% decreased risk for developing *Aspergillus* colonization after transplantation, which was dose-dependent during the first year post-transplant. These findings are particularly notable as post-transplant *Aspergillus* colonization has been associated with up to a 11-fold increased risk of developing invasive aspergillosis as well as a 2-fold increased risk of all-cause mortality; even among individuals who never develop invasive aspergillosis.[32–34]

Indeed, voriconazole exposure reduced mortality by 66% among those with *Aspergillus* colonization, possibly due to prevention of other sequelae of colonization, such as bronchiolitis obliterans syndrome,[35,36] even in the absence of a statistically significant reduction of invasive aspergillosis. This relationship was not dose-dependent, however, suggesting that prolonged voriconazole administration may not provide significant mortality benefit compared to shorter treatment durations in these subjects.

Individuals without evidence of *Aspergillus* colonization appear particularly susceptible to an imbalance in the risk and benefits of voriconazole administration. In those without colonization, voriconazole conferred no risk reduction in either invasive aspergillosis or mortality. In fact, we found a 2% *increased* risk of mortality for each additional 30-days of exposure. An alternative possibility is that individuals receiving higher cumulative doses of voriconazole possess additional risk factors for death that were not controlled for in our models, such as increased intensity of immunosuppression[37] or episodes of rejection.[38] Prior studies have not reported this relationship, but this deserves further investigation.[39]

Efficacy data concerning the benefits of voriconazole prophylaxis in lung transplant recipients still remains limited. A recent systematic review and meta-analysis of universal antifungal prophylaxis in lung transplantation concluded that there is no definitive evidence that universal antifungal prophylaxis, including regimens that utilize voriconazole, significantly reduce incidence of invasive aspergillosis.[40] Of the studies that have suggested that voriconazole prophylaxis prevents invasive aspergillosis and death[10], most have assessed voriconazole prophylaxis only administered in the immediate post-transplant period (i.e. 1–6 months after lung transplantation) and primary outcomes only until 12 months after transplant.[41] Yet, many patients receive much longer durations of voriconazole exposure related to additional targeted prophylaxis in the setting of increased immunosuppression for allograft rejection or for treatment of an fungal colonization or an invasive infection. It may be that the benefits of voriconazole prophylaxis in the immediate post-transplant period do not extend to long-term benefit. A multi-center, randomized clinical trial is still needed to determine the true efficacy of voriconazole prophylaxis in lung transplant recipients.[39]

Our study has particular strengths. It is the first study to assess risk for voriconazole-associated SCC in lung transplant recipients weighed against the benefits of reducing invasive aspergillosis and death. As a 21-year, single center retrospective cohort study, we had access to detailed medical records for all cohort members including specific dates and duration of drug exposure in temporal relation to development of SCC, *Aspergillus* colonization, invasive aspergillosis, and death. Primary outcomes were screened and

identified by ICD-9 code, but also confirmed by defined pathologic or clinical criteria. We used statistically rigorous methods and adjusted our models for a number of known risk factors for our primary outcomes. In addition, our expanded cohort of 455 lung transplant recipients significantly increased our study power compared to our previous work and validated our prior findings.[16]

This study also had limitations. First, there were a number of confounding risk factors that were not controlled for in our regression models. However, we believe that we included and controlled for major confounders. Due to incomplete data, we were unable assess smoking history which is a known risk factor for SCC.[20] Additionally, we were unable to retrospectively obtain the Fitzpatrick skin type or relative sun exposure of cohort members. Despite this limitation, we believe it is unlikely that these variables differed by voriconazole exposure. Second, we only captured outpatient voriconazole administration and did not assess inpatient exposure. Thus, we may have overestimated the relative impact of each unit of voriconazole exposure in our cumulative dose models. Third, the EORTC criteria that we used to define cases of invasive aspergillosis may overestimate cases of invasive fungal infections in the lung transplant recipient patient population. This is because there is a more heterogeneous range of radiologic findings in lung transplant patients with or without true invasive fungal infections after transplant. However, we used a standard definition used in other lung transplant studies.[42,43] We do not believe misclassification of this outcome applies to a large proportion of our cases and that, if such misclassification did occur, it would likely be non-differential by voriconazole exposure status and would only bias our results towards the null.

Finally, we were unable to control for relative type and intensity of immunosuppression. At UCSF, maintenance immunosuppression has typically been accomplished with a combination of anti-proliferative agents, calcineurin inhibitors, and systemic steroids. In the late 1990s, our routine choice for calcineurin inhibitors switched from cyclosporine to tacrolimus. In 2004, our choice for antiproliferative agent switched from azathioprine to mycophenolate mofetil. Our use of corticosteroid dosing has not change substantially over the last two decades. Analytically, we included year of transplant (era effect) in our multivariate analysis which may have accounted for differences in immunosuppression that varied over 21-year the study period. In fact, inclusion of this variable did have a significant impact on select analyses, particularly those assessing the relationship between voriconazole exposure and all-cause mortality. While there is no universally accepted measure of relative level of immunosuppression, future studies could incorporate assessments of indicators including episodes of rejection, type, dose, and duration of immunosuppression, and other heterogeneous health and immune-related phenotypes to better control for this variable..

In summary, we found that voriconazole exposure is independently associated with risk for SCC in lung transplant recipients, that this relationship is dose-dependent, and that it is specific to voriconazole among the antifungals examined. Individuals with older age at transplant, white race, and male sex were the most at risk. We also found that voriconazole exposure significantly reduced risk of developing *Aspergillus* colonization, especially during the first-year post transplant. Voriconazole exposure significantly reduced all-cause

mortality, but this benefit was limited to those who developed post-transplant *Aspergillus* colonization.

These findings have important implications for the care of lung transplant recipients. SCCs in solid organ transplant recipients behave aggressively and can result in significantly reduced quality of life, metastatic disease, and increased mortality.[2,44] It is important for physicians to be aware of the impact of voriconazole on these outcomes. As such, we recommend all providers counsel lung transplant recipients on skin cancer education and photoprotection, in addition to scheduling routine skin cancer screening with a trained dermatologist after transplantation[8] Lung transplant programs should also consider patient specific risk factors when deciding on the type, dose, and duration of antifungal prophylaxis regimens. In particular, the risks and benefits of voriconazole prophylaxis should be weighed carefully. Among lung transplant recipients with risk factors for SCC, including those with older age, male sex, and white race, or among those whom prolonged voriconazole administration may not have clear benefit, including subjects lacking evidence of *Aspergillus* colonization, transplant physicians should consider limiting exposure to high doses of voriconazole or utilizing alternative pharmacologic options that do not pose an increased risk for SCC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

MM is supported by the Stanford University Medical Scholars Research Grant. PCH is supported by the National Center for Advancing Translational Sciences, National Institutes of Health, through UCSF-CTSI Grant Numbers UL1 TR000004 and TL1 TR000144. JS is supported by the NIH/National Heart, Lung, and Blood Institute, through Grant Number K23 HL111115. SA is supported by UCSF-CTSI Grant Number KL2 TR000143. This study was supported by the UCSF Nina Ireland Lung Disease Program. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the U.S. Department of Health and Human Services. The funding agencies played no role in the collection, analysis, interpretation, or publication of this data.

Abbreviations

SCC	Squamous Cell Carcinoma
OPTN	Organ Procurement and Transplantation Network
HR	Unadjusted Hazard Ratio
aHR	Adjusted Hazard Ratio
eHR	Expanded Hazard Ratio
SD	Standard Deviation
CI	Confidence Interval

References

1. Berg D, Otley C. Skin cancer in organ transplant recipients: Epidemiology, pathogenesis, and management. J Am Acad Dermatol. 2002; 47:1–17. [PubMed: 12077575]

2. Sheil AG, Disney AP, Mathew TH, Amiss N. De novo malignancy emerges as a major cause of morbidity and late failure in renal transplantation. Transplant Proc. 1993; 25(I.2):1383–1384. [PubMed: 8442150]

3. Lindelof B, Jarnvik J, Ternesten-Bratel A, Granath F, Hedblad MA. Mortality and clinicopathological features of cutaneous squamous cell carcinoma in organ transplant recipients: a study of the Swedish cohort. Acta Derm Venereol. 2006; 86(3):219–222. [PubMed: 16710578]

4. Ramsay HM, Fryer AA, Reece S, Smith AG, Harden PN. Clinical risk factors associated with nonmelanoma skin cancer in renal transplant recipients. Am J Kidney Dis. 2000; 36:167–74. [PubMed: 10873887]

5. Roeger LS, Sheil AGR, Disney APS, Mathew TH, Amiss N. Risk factors associated with the development of squamous cell carcinomas in immunosuppressed renal transplant recipients. Clin Transplant. 1992; 6:202–216.

6. Jensen P, Hansen S, Moller B, Leivestad T, Pfeffer P, Geiran O, et al. Skin cancer in kidney and heart transplant recipients and different long-term immunosuppressive therapy regimens. J Am Acad Dermatol. 1999; 40(2):177–186. [PubMed: 10025742]

7. Jensen P, Hansen S, Moller B, Leivestad T, Pfeffer P, Fauchald P. Are renal transplant recipients on CsA-based immunosuppressive regimens more likely to develop skin cancer than those on azathioprine and prednisolone? Transplant Proc. 1999; 31(1–2):1120. [PubMed: 10083500]

8. Zwald FO, Brown M. Skin cancer in solid organ transplant recipients: advances in therapy and management: part 1. Epidemiology of skin cancer in solid organ transplant recipients. J Am Acad Dermatol. 2011; 65(2):253–261. [PubMed: 21763561]

9. Sole A, Morant P, Salavert M, Peman J, Morales P. Aspergillus infections in lung transplant recipients: risk factors and outcome. Clin Microbiol Infect. 2005; 11:359–365. [PubMed: 15819861]

10. Husain S, Paterson D, Studer S, Pilewski J, Crespo M, Zaldonis D, et al. Voriconazole prophylaxis in lung transplant recipients. Am J Transplant. 2006; 6(12):3008–3016. [PubMed: 17062003]

11. Neoh CF, Snell GI, Kotsimbos T, et al. Antifungal prophylaxis in lung transplantation – a world-wide survey. Am J Transplant. 2011; 11(2):361–366. [PubMed: 21272239]

12. Herbrecht R, Denning D, Patterson T, Bennett J, Greene R, Oestmann J, et al. Voriconazole versus Amphotericin B for Primary Therapy of Invasive Aspergillosis. New England Journal of Medicine. 2002; 347(6):408–415. [PubMed: 12167683]

13. Feist A, Lee R, Osborne S, Lane J, Yung G. Increased incidence of cutaneous squamous cell carcinoma in lung transplant recipients taking long-term voriconazole. J Heart Lung Transplant. 2012; 31:1177–1181. [PubMed: 22841559]

14. Vadnerkar A, Nguyen M, Mitsani D, Crespo M, Pilewski J, Toyoda Y, et al. Voriconazole exposure and geographic location are independent risk factors for squamous cell carcinoma of the skin among lung transplant recipients. J Heart Lung Transplant. 2010; 29(11):1240–1244. [PubMed: 20591690]

15. Zwald F, Spratt M, Lemos B, Veledar E, Lawrence C, Marshall Lyon G, et al. Duration of voriconazole exposure: an independent risk factor for skin cancer after lung transplantation. Dermatol Surg. 2012; 38(8):1369–1374. [PubMed: 22551390]

16. Singer J, Boker A, Metchnikoff C, Binstock M, Boettger R, Golden J, et al. High cumulative dose exposure to voriconazole is associated with cutaneous squamous cell carcinoma in lung transplant recipients. J Heart Lung Transplant. 2012; 31:694–9. [PubMed: 22484291]

17. Voriconazole [package insert]. New York: Pfizer; 2009.

18. Epaulard O, Villier C, Ravaud P, Chosidow O, Blanche S, Mamzer-Bruneel M, et al. A Multistep Voriconazole-Related Phototoxic Pathway May Lead to Skin Carcinoma: Results From a French Nationwide Study. Clinical Infectious Diseases. 2013; 57(12):e182–e188. [PubMed: 24046296]

19. Egan T, Murray S, Bustami R, Shearon T, McCullough K, Edwards L, et al. Development of the New Lung Allocation System in the United States. Am J Transplant. 2006; 6(5p2):1212–1227. [PubMed: 16613597]

20. De Hertog S, Wensveen C, Bastiaens MT, Kielich C, Berkhout M, Westendorp R, et al. Relation between smoking and skin cancer. Journal of Clinical Oncology. 2001; 19(1):231–238. [PubMed: 11134217]

21. Kotton C, Kumar D, Caliendo A, Åsberg A, Chou S, Danziger-Isakov L, et al. Updated International Consensus Guidelines on the Management of Cytomegalovirus in Solid-Organ Transplantation. Transplantation Journal. 2013; 96(4):333–360.
22. Wiita AP, Roubinian N, Khan Y, et al. Cytomegalovirus disease and infection in lung transplant recipients in the setting of planned indefinite valganciclovir prophylaxis. Transpl Infect Dis. 2012; 4(3):248–58. [PubMed: 22385394]
23. Binder DA. Fitting Cox proportional hazards models from survey data. Biometrika. 1992; 79(1): 139–147.
24. Lin DY. On fitting Cox's proportional hazards models to survey data. Biometrika. 2000; 87(1):37–47.
25. Zwald FO, Brown M. Skin cancer in solid organ transplant recipients: Advances in therapy and management: part 1. Epidemiology of skin cancer in solid organ transplant recipients. J Am Acad Dermatol. 2011; 65:253–261. [PubMed: 21763561]
26. Luong M, Chaparro C, Stephenson A, Rotstein C, Singer L, Waters V, et al. Pretransplant Aspergillus Colonization of Cystic Fibrosis Patients and the Incidence of Post–Lung Transplant Invasive Aspergillosis. Transplantation Journal. 2014; 97(3):351–357.
27. Döring M, Blume O, Haufe S, Hartmann U, Kimmig A, Schwarze C, et al. Comparison of itraconazole, voriconazole, and posaconazole as oral antifungal prophylaxis in pediatric patients following allogeneic hematopoietic stem cell transplantation. Eur J Clin Microbiol Infect Dis. 2013; 33(4):629–638. [PubMed: 24173819]
28. O'Sullivan A, Pandya A, Papadopoulos G, Thompson D, Langston A, Perfect J, et al. Cost-Effectiveness of Posaconazole versus Fluconazole or Itraconazole in the Prevention of Invasive Fungal Infections among Neutropenic Patients in the United States. Value in Health. 2009; 12(5): 666–673. [PubMed: 19508661]
29. McLaughlin J, Equils O, Somerville K, Aram J, Schlamm H, Welch V, et al. Risk-adjusted relationship between voriconazole utilization and non-melanoma skin cancer among lung and heart/lung transplant patients. Transplant Infectious Disease. 2013; 15(4):329–343. [PubMed: 23489832]
30. Gray D. Trends in the population-based incidence of squamous cell carcinoma of the skin first diagnosed between 1984 and 1992. Archives of Dermatology. 1997; 133(6):735–740. [PubMed: 9197827]
31. Armstrong B, Kricker A. The epidemiology of UV induced skin cancer. Journal of Photochemistry and Photobiology B: Biology. 2001; 63(1–3):8–18.
32. Luong M, Chaparro C, Stephenson A, Rotstein C, Singer L, Waters V, et al. Pretransplant Aspergillus Colonization of Cystic Fibrosis Patients and the Incidence of Post–Lung Transplant Invasive Aspergillosis. Transplantation Journal. 2014; 97(3):351–357.
33. Cahill B. Aspergillus Airway Colonization and Invasive Disease After Lung Transplantation. CHEST. 1997; 112(5):1160. [PubMed: 9367451]
34. Felton T, Roberts S, Isalska B, Brennan S, Philips A, Whiteside S, et al. Isolation of Aspergillus species from the airway of lung transplant recipients is associated with excess mortality. Journal of Infection. 2012; 65(4):350–356. [PubMed: 22863902]
35. Garantziotis S, Palmer S. An Unwelcome Guest: Aspergillus Colonization in Lung Transplantation and Its Association with Bronchiolitis Obliterans Syndrome. American Journal of Transplantation. 2009; 9(8):1705–1706. [PubMed: 19538485]
36. Weigt S, Copeland C, Derhovanessian A, Shino M, Davis W, Snyder L, et al. Colonization With Small Conidia Aspergillus Species Is Associated With Bronchiolitis Obliterans Syndrome: A Two-Center Validation Study. Am J Transplant. 2013; 13(4):919–927. [PubMed: 23398785]
37. Singh N, Husain S. Aspergillosis in Solid Organ Transplantation. Am J Transplant. 2013; 13(s4): 228–241. [PubMed: 23465016]
38. Verleden G, Vos R, Verleden S, De Wever W, De Vleeschauwer S, Willems-Widyastuti A, et al. Survival Determinants in Lung Transplant Patients With Chronic Allograft Dysfunction. Transplantation. 2011; 92(6):703–708. [PubMed: 21836537]
39. Clancy C, Nguyen M. Long-term voriconazole and skin cancer: is there cause for concern? Curr Infect Dis Rep. 2011; 13(6):536–543. [PubMed: 21997681]

40. Bhaskaran A, Mumtaz K, Husain S. Anti-Aspergillus Prophylaxis in Lung Transplantation: A Systematic Review and Meta-analysis. Curr Infect Dis Rep. 2013; 15(6):514–525. [PubMed: 24173585]

41. Schaenman J. Is universal antifungal prophylaxis mandatory in lung transplant patients? Curr Opin Infect Dis. 2013; 26(4):317–325. [PubMed: 23743815]

42. Luong M, Hosseini-Moghaddam S, Singer L, et al. Risk factors for voriconazole hepatotoxicity at 12 weeks in lung transplant recipients. Am J Transplant. 2012; 12(7):1929–35. [PubMed: 22486950]

43. Doligalski C, Benedict K, Cleveland A, et al. Epidemiology of invasive mold infections in lung transplant recipients. Am J Transplant. 2014; 14(6):1328–33. [PubMed: 24726020]

44. Ong C, Keogh A, Kossard S, et al. Skin cancer in Australian heart transplant recipients. J Am Acad Dermatol. 1999; 40(1):27–34. [PubMed: 9922009]

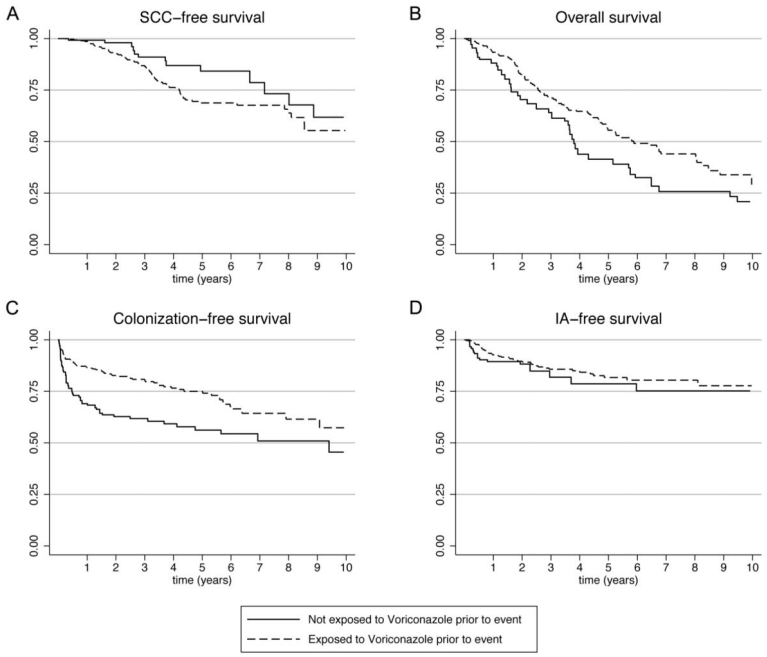

Figure 1.

Unadjusted Kaplan-Meier plots of (A) Squamous Cell Carcinoma (SCC)-free survival (B) Overall survival (C) Aspergillus colonization-free survival, and (D) Invasive Aspergillosis-free survival, stratified by “ever” (exposed, dashed lines) versus “never” (not exposed, solid lines) voriconazole exposure prior to development of each primary outcome over time (years) after lung transplantation.

Table 1

Cohort Demographic Characteristics

Characteristic[a]	Lung Transplant Cohort (n=455)
Age at transplant, years	52.4 ± 12.4
Age <50	164 (36.0)
Age ≥ 50	291 (64.0)
Sex	
Male	244 (53.6)
Female	211 (46.4)
Race/Ethnicity	
White, non-Hispanic	360 (79.1)
Non-White	95 (20.9)
Black	26 (5.7)
Hispanic	42 (9.2)
Asian	20 (4.4)
Am Indian/ Alaskan Native	3 (0.7)
Hawaiian/Other	3 (0.7)
Multiracial	1 (0.2)
LT indication by diagnostic category[b]	
Group A (COPD)	141 (31.0)
Group B (Pulmonary Hypertension)	44 (9.7)
Group C (Cystic Fibrosis)	48 (10.6)
Group D (Pulmonary Fibrosis)	222 (48.8)
Type of Transplant	
Single Lung	98 (21.5)
Bilateral Lung	347 (76.3)
Heart-Lung	10 (2.2)
Year of Transplant	2005 (5.4)

[a] Continuous data are presented as mean +/− standard deviation, and categorical data as number (%).

[b] Lung Allocation Score (LAS) diagnostic category

Table 2

Unadjusted, Adjusted, and Expanded Hazard Ratios for Primary and Secondary Outcomes, by Voriconazole Exposure

	Unadjusted HR (95% CI)	p-value	Adjusted HR (aHR)[a] (95% CI)	p-value	Expanded HR (eHR)[b] (95% CI)	p-value
Squamous Cell Carcinoma						
Any Exposure[c]	1.91 (1.11–3.27)	0.02	1.73 (1.04–2.88)	0.03	1.71 (0.83–3.52)	0.15
Cumulative Dose Exposure[d]	1.02 (1.01–1.03)	0.001	1.03 (1.02–1.04)	<0.001	1.03 (1.02–1.04)	<0.001
Aspergillus Colonization						
Any Exposure[c]	0.52 (0.39–0.71)	<0.001	0.52 (0.38–0.70)	<0.001	0.50 (0.34–0.72)	<0.001
Cumulative Dose Exposure[d]	0.99 (0.96–1.01)	0.34	0.99 (0.96–1.01)	0.37	1.00 (0.97–1.02)	0.71
Invasive Aspergillosis						
Any Exposure[c]	0.76 (0.43–1.35)	0.35	0.79 (0.45–1.37)	0.40	0.78 (0.40–1.49)	0.45
Cumulative Dose Exposure[d]	0.98 (0.95–1.02)	0.32	0.98 (0.95–1.02)	0.33	0.98 (0.95–1.02)	0.38
All-Cause Mortality						
Any Exposure[c]	0.66 (0.47–0.93)	0.02	0.64 (0.45–0.90)	0.01	1.32 (0.87–1.99)	0.19
Cumulative Dose Exposure[d]	1.01 (1.00–1.02)	0.04	1.01 (1.01–1.02)	0.05	1.02 (1.01–1.03)	<0.001

[a] Adjusted for sex, race (White vs. Non-White) and age at transplant

[b] Adjusted for sex, race (White vs. Non-White), age at transplant and year transplant

[c] Versus subjects never exposed to voriconazole

[d] Per 12 grams of voriconazole exposure (equivalent to 200 mg BID for 30 days).

Table 3

Adjusted Cumulative Incidence of Primary Outcomes, by Voriconazole Exposure

	Adjusted Cumulative Incidence[a]		
Years Post-Transplant	**"Never" Voriconazole Exposure (−)**	**"Ever" Voriconazole Exposure (+)**	**Absolute Risk Difference**
Squamous Cell Carcinoma			
1 year	1%	1%	0%
5 years	15%	25%	+ 10%
10 years	28%	43%	+ 15%
Aspergillus Colonization			
1 year	28%	15%	−13%
5 years	44%	25%	−19%
10 years	63%	39%	−24%
Invasive Aspergillosis			
1 year	10%	8%	− 2%
5 years	22%	18%	− 4%
10 years	27%	21%	− 6%
All Cause Mortality			
1 year	7%	8%	+1%
5 years	39%	47%	+8%
10 years	58%	69%	+11%

[a] Adjusted for age at transplant, sex, race, and year of transplant.