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Cutaneous Squamous Cell Carcinomas in Solid Organ Transplant Recipients

A Thesis Submitted to the  
Yale University School of Medicine  
in Partial Fulfillment of the Requirements for the  
Degrees of  
Doctor of Medicine  
and  
Master of Health Science

By  
Joyce Y. Cheng  
2017

## CUTANEOUS SQUAMOUS CELL CARCINOMA IN SOLID ORGAN TRANSPLANT RECIPIENTS: A SINGLE-CENTER EXPERIENCE

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Solid organ transplant recipients (SOTR) have an approximately 100-fold increased risk of developing cutaneous squamous cell carcinoma (SCC). These SCCs may behave more aggressively than SCCs developing in non-immunosuppressed individuals. The purpose of this study was to gather more data regarding aggressive behavior of SCCs in immunosuppressed SOTRs compared with SCCs occurring in an immunocompetent control group. An 8-year retrospective observational cohort study was conducted to compare the demographics, care received by, and outcomes of 98 adult SOTR and immunocompetent patients with at least one histopathologically confirmed SCC. The two groups were statistically comparable with regards to demographics, patient care, follow-up time, and numbers of skin lesions they developed, though the SOTR group had significantly higher annual visit frequency (4 office visits per patient per year vs. 3,  $p = 0.025$ ) and annual biopsy rates (6 biopsies per patient per year vs. 5,  $p = 0.039$ ). In this setting, the SCCs developed by SOTRs did not appear to be significantly more aggressive than those in the immunocompetent control group. Our SOTRs did not develop significantly thicker tumors than the immunocompetent controls. One SOTR developed an SCC with perineural invasion, two SOTRs had regional metastasis, and one SOTR had nodal metastasis. An increased risk of carcinogenesis with increasing cumulative years of immunosuppression was surprisingly not observed among the SOTRs. SOTRs had a 90% greater risk of developing SCCs in the head and neck region compared with the immunocompetent group (RR=1.89, 95% CI 1.04-1.37). Taken together, our findings suggest that the drastically increased risk of SCCs in SOTRs and potential for aggressive behavior may be successfully managed to a level comparable to that in high-risk immunocompetent individuals with close adherence to current dermatologic surveillance recommendations for SOTRs and a marginally lower threshold for biopsy of suspicious lesions.

## **Acknowledgments**

To Dr. Colegio,

For your outstanding mentorship and for believing in me.

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## Introduction

### 1. Long-term outcomes lag behind advances in short-term outcomes in organ transplantation

On January 9, 2017, the U.S. Organ Procurement and Transplantation Network announced that a record-breaking 33,606 organ transplants were performed in 2016, driven largely by an increase in the number of deceased donors. Since 1988, more than 650,000 organs have been transplanted. Approximately 120,000 patients with end-stage organ failure currently await organ transplantation.<sup>1</sup> As the number of transplants performed increases each year and post-operative outcomes and short-term survival improve, the population of solid organ transplant recipients (SOTRs) living in the United States has also grown steadily.

Unfortunately, long-term SOTR outcomes still lag behind these remarkable advances in short-term post-transplantation outcomes.<sup>2,3</sup> Lodhi *et al.* examined long-term graft failure rates of SOTRs over a 20-year period and found minimal improvement in annual graft failure rates 5 and 10 years out from transplant; failure rates in liver transplant patients incrementally decreased from 4.7% to 4.3%, while in heart transplant patients, they trended down from 6.4% to 5.1%.<sup>2</sup> Significant improvement in these long-term outcomes has largely been limited by lack of a way to specifically and sustainably induce immunologic non-responsiveness or operational tolerance to donor alloantigens. As a result, SOTRs must take immunosuppressive drugs for the lifetime of their graft in order to maintain allograft function.<sup>4</sup>

Chronic exposure to immunosuppressive drugs in SOTRs is associated with a multitude of potential complications, of which medical providers should be cognizant in seeking to optimize SOTR health. Besides increased risks of cardiovascular disease and

infection, chronic immunosuppressive therapy is also associated with a substantially increased risk of diverse cancers, including non-Hodgkins lymphoma and Kaposi's sarcoma.<sup>5-7</sup> A large, population-based study of US transplant recipients found that SOTRs have an overall 2-fold greater risk of malignancy compared with the general population, including both infection-related and unrelated malignancies.<sup>8,9</sup> Studies in transplant populations around the world independently report increased risk of malignancy following transplantation ranging from a 2- to 4-fold increase compared with the general population.<sup>10-17</sup>

While significant progress has been made in managing the elevated risks of death from cardiovascular disease and infection in SOTRs with aggressive screening, prophylaxis, and interventional therapies, malignancy remains an important and challenging cause of morbidity and mortality in SOTRs with a functional graft.<sup>4,7,18</sup> Not only are SOTRs more likely to develop malignancy compared with the general population, they are also more likely to succumb to their malignancy, regardless of age, sex, transplanted organ, or transplant period.<sup>19-21</sup> Possible reasons include increased aggressiveness of neoplasms arising in an immunosuppressed host and reluctance on the part of the transplant clinician to pursue intensive treatment of the cancer due to a patient's medical comorbidities and fear of allograft rejection and loss.<sup>18,21</sup> Paying close attention to cancer prevention and screening recommendations in the care of SOTRs is crucial to improve long-term post-transplantation outcomes.<sup>3,5,6</sup>

## 2. Skin cancer causes significant morbidity and mortality among solid organ transplant recipients

While SOTRs have an overall increased risk of cancer compared with the general

population, they experience a particularly marked increase in the risk of all types of skin cancers. Precise estimates of risk relative to the general population have been difficult to make, especially given that most national cancer registries do not record skin cancers. However, it is estimated that SOTRs have an up to 3-fold increase in the risk of malignant melanoma, an up to 10-fold increase in the risk of basal cell carcinoma (BCC), and an up to 100-fold increase in the risk of squamous cell carcinoma (SCC).<sup>22-25</sup>

A recent population-based, multi-center cohort study of 10,460 SOTRs reported that the overall incidence rate of skin cancer in SOTRs (IR 1,437 per 100,000 person-years) is almost 5-fold greater than the rate of all cancers combined in the general US population. Most of these cancers were SCCs (IR 1355 per 100,000 person-years). Several risk factors were identified in the study as elevating this risk further. The incidence rate of skin cancer in white patients was 22-fold greater than in nonwhite patients, and the risks of skin cancer in men, patients older than 50 years of age, and thoracic organ transplant recipients were between 1.7- to 3-fold greater than in women, patients younger than 50 years of age, and abdominal transplant recipients.<sup>26</sup>

Skin cancer after transplantation has also been associated with increased mortality. For reference, about 1.5% to 2.1% of cutaneous SCCs result in death in the general population.<sup>27</sup> A study of all U.S. SOTRs between 1987 and 2013 reported a skin cancer-related mortality rate (HR 35.27 per 100,000 person-years) nearly 9-fold greater than that reported in the general population, with particularly increased mortality occurring in white patients, male patients, patients with thoracic organ transplants, patients with increased post-transplant survival time, and patients older than 50 years of age.<sup>28</sup>



### 3. Factors contributing to the greatly increased risk of cutaneous squamous cell carcinomas in solid organ transplant recipients

A synergistic combination of immunosuppression, viral infection, environmental ultraviolet (UV) radiation, and the mechanisms of action of immunosuppressive medications is thought to beget the drastically increased SCC risk in SOTRs.<sup>29,30</sup>

Increased skin cancer risk in patients with acquired immunodeficiency syndrome (AIDS) supports a contribution of immunosuppression and viral infection to increased skin cancer risk in SOTRs.<sup>13</sup> The immune system is involved both in the control of oncogenic viruses such as human papillomavirus (HPV) and in tumor cell surveillance, and iatrogenic immunosuppression of SOTRs to prevent graft rejection could impair these anti-tumor functions, leading to tumor cell proliferation.<sup>31</sup> However, the increased skin cancer risk in patients with AIDS does not begin to approach that in SOTRs; one study found the risk of NMSC among its subjects with AIDS to be 4.11 (95% CI 1.08-16.6), while the risk of NMSC among its SOTR subjects was 28.62 (95% CI 9.39-87.2).<sup>13</sup> This suggests that immunosuppression and viral infection cannot fully account for the magnitude of the SCC risk in SOTRs.

Several studies independently associate Fitzpatrick skin type, sun exposure history, and skin cancer risk in SOTRs.<sup>32,33</sup> A study from the United Kingdom observed a 15% incidence of NMSC in SOTRs of African ancestry compared with a 26% overall 10-year incidence of NMSC among all of the SOTRs in its study cohort.<sup>34</sup> That SOTRs with more skin pigmentation had somewhat decreased NMSC risk compared to less pigmented counterparts supports a contribution of UV radiation in the development of skin cancers in SOTRs. However, environmental UV exposure, immunosuppression, and viral infection still do not fully explain the magnitude of the increased SCC risk in

SOTRs.

The immunosuppressive agents SOTRs must take to maintain function of their graft may round out the picture of exceptionally increased SCC risk in SOTRs. Numerous studies have shown that the heightened risk of skin cancers increases with greater cumulative time on immunomodulatory therapy and with increasing intensity of the regimen; for example, heart and lung transplant recipients who require more intensive immunosuppressive effects also experience greater tumor risk.<sup>35</sup> Choice of immunosuppressive medication matters. One study found that patients on the antimetabolite azathioprine may be more than twice as likely to develop SCC (OR 2.67, 95% CI 1.23-5.76).<sup>36</sup> Multiple clinical trials have shown that switching SOTRs from calcineurin inhibitors to mammalian target of rapamycin (mTOR) inhibitors consistently decreases incidence of new skin cancers in SOTRs.<sup>29</sup>

Calcineurin inhibitors commonly used in immunosuppressive regimens such as cyclosporine have been shown to interfere with p53 signaling and nucleotide excision repair.<sup>37</sup> Calcineurin and NF-AT signaling are vital for p53-mediated senescence; aberrant p53 signaling is observed in SCCs caused by UVR mutations, arsenic, and HPV.<sup>38,39</sup> Calcineurin inhibitors interfere with nucleotide excision repair by down-regulating transcription of xeroderma pigmentosum complementation group A and xeroderma pigmentosum complementation group G, impairing cells' abilities to repair UV-induced DNA damage.<sup>40</sup> It is no wonder that calcineurin inhibitors increase risk of SCCs in SOTRs, especially in the presence of UV exposure, given that their mechanism of action synergistically impairs at least two anti-tumor pathways.

In contrast, mTOR inhibitors such as sirolimus have been shown in mouse models to restrain both immune function and proliferation in cancer cells.<sup>41</sup> In all clinical trials

comparing use of calcineurin inhibitors with mTOR inhibitors in SOTRs, no difference in graft rejection rates were observed. Unfortunately, use of sirolimus in SOTRs is limited in practice by the incidence of serious adverse effects, including pneumonitis and peripheral edema, and patients should be advised to discuss the risks and benefits of switching to sirolimus with their transplant specialist.<sup>29</sup>

#### 4. Epidemiology and distinct presentation of cutaneous squamous cell carcinomas in solid organ transplant recipients

As mentioned in the previous section, it has been estimated that SOTRs have a dramatic up to 100-fold increase in the risk of cutaneous squamous cell carcinoma (SCC) compared with the general population.<sup>22-25</sup> The ratio of SCC to BCC in SOTRs is approximately 4 to 1, an inversion of the ratio seen in the general population.<sup>42</sup> The incidence rate of SCC (IR 1355 per 100,000 person-years) essentially drove the overall incidence rate of skin cancer in SOTRs in a multi-center, population-based study.<sup>26</sup>

Cutaneous SCCs tend to present at a younger age overall in SOTRs, typically 3-8 years after transplant.<sup>42,43</sup> Data from single-center studies suggest that the interval between transplantation and SCC diagnosis may correlate with the age of the patient at transplant. For example, Webb *et al.* reported that the mean interval between transplantation and diagnosis of a tumor in 1,067 subjects transplanted after the age of 60 was three years at their institution,<sup>44</sup> while Euvrard *et al.* found that the mean interval between transplantation and diagnosis of a tumor for patients who were transplanted in their 40s was eight years.<sup>45</sup>

Most SOTRs will develop a few SCCs. One Scandinavian study found that 50% of patients with a first SCC will be diagnosed with a second one within 3.5 years.<sup>46</sup>

Severity of SCCs is linked to their number; a significant subgroup of SOTRs may develop dozens to hundreds of distinct SCCs over time, primarily in sun-exposed areas.<sup>46</sup> Euvrard *et al.* observed an age-related difference in the anatomic distribution of SCCs; they found that the majority of lesions in patients under the age of 40 at transplant were located on the dorsum of the hands, forearms, or upper trunk, while the majority of lesions in older SOTRs were located on the head.<sup>45</sup>

SCCs developing in SOTRs may also differ histologically from those occurring in non-immunosuppressed individuals. Smith *et al.* examined 601 primary SCCs in 518 immunocompetent individuals and 231 primary SCCs in 79 SOTRs—53 kidney transplant patients, 25 heart or lung transplant patients, and 1 liver transplant recipient. Histologic features they observed significantly more often in SOTRs included acantholytic changes, early dermal invasion, an infiltrative growth pattern with or without desmoplasia, Bowen's disease with carcinoma, and a significant angiogenic vascular component. The thickness of SCCs in the SOTR group was found to be significantly greater than that in the immunocompetent group.<sup>47</sup> Their study did not correlate these tumor characteristics with prognosis.

However, SCCs in SOTRs can have an aggressive clinical course and should be treated by physicians as being prone to recur locally and to metastasize. Commonly cited figures in the literature for the local recurrence rate of SCCs in SOTRs in the first six months after excision, 13.4%, and for lymph node metastasis, 5-8%, during the second year after excision,<sup>42</sup> come from a Dutch study of 1,546 renal transplant patients. Ten (14.1%) of 71 patients in this study who developed one or more SCCs experienced local recurrence after a mean period of 19 months. In their study, 1 patient eventually succumbed to dedifferentiated carcinoma.<sup>48</sup>

In the presence of certain risk factors such as perineural invasion, which is found in 2.5% to 14% of SCCs in the general population, these risks appear to be even greater.<sup>49,50</sup> Local recurrence occurs in as many as 16% to 45% of SCCs, while nodal metastasis occurs in as many as 10% to 50% of SCCs.<sup>51,52</sup> It is reasonable to assume that, in the presence of perineural invasion, SCC outcomes could be worse in high-risk immunocompromised SOTRs.

Metastatic skin cancers in SOTRs have poor prognosis. A multicenter, international study assembled a cohort of 73 metastatic skin cancers in SOTRs, 85% of which originated from a primary SCC, to better study the clinical course, treatment, and final outcome in this rare occurrence. They found a 3-year disease-specific survival of 56%. The mean time from transplant to diagnosis of first metastasis was 10.7 years among the patients in their cohort. After diagnosis of the primary tumor, however, metastases developed rapidly within a mean of 1.4 years, underscoring the importance of close clinical follow-up by dermatologists after a high-risk primary tumor has been diagnosed in SOTRs.<sup>53</sup>

##### 5. Cutaneous squamous cell carcinomas can behave aggressively in solid organ transplant recipients

Cutaneous squamous cell carcinomas are generally thought to be more aggressive in SOTRs than in non-immunosuppressed persons.<sup>42</sup> However, the literature from which this assumption of aggressiveness has been drawn is not entirely conclusive, composed of different studies examining isolated indicators of aggressive behavior or of methodologically limited studies. In addition, there is a lack of consensus in the literature about the definition of aggressiveness.

In a study of head and neck tumors including cutaneous tumors in cardiothoracic transplant recipients done at Stanford, aggressiveness was defined as being associated with metastatic disease, requiring extensive radiation therapy, chemotherapy, or radical surgical intervention, or directly causing death. Thirty tumors (13.3%) of a cohort of 214 cutaneous SCCs and 55 BCCs behaved aggressively according to this definition among their study population. Unfortunately, a more detailed breakdown of aggressiveness by tumor subtype was not reported by the investigators, so the relative proportion of SCCs behaving aggressively cannot be parsed out. Metastatic cutaneous SCC was reported as the second leading cause of death behind post-transplant lymphoproliferative disorder, accounting for 7 (22.5%) of 31 cancer-related deaths in their study population.<sup>54</sup>

An Australian study of 619 cardiothoracic transplant recipients defined an aggressive cutaneous malignancy as having any of the following features—local invasion and/or regional metastases at diagnosis, poor differentiation, and locoregional and/or systemic relapse following therapy. Nineteen of their subjects (3.1%) developed 20 SCCs fitting their stated criteria for aggressiveness, and poor histologic differentiation was found to be associated with aggressive behavior. At two years, 66% of the cohort had either died of the disease or experienced metastasis.<sup>55</sup>

Lott *et al.* set out to examine this question more definitively by comparing 153 SOTRs with 978 documented SCCs against 154 control patients with 256 documented SCCs and evaluating their tumors for factors of aggressiveness. Aggressiveness was defined in terms of local recurrence rate, lymph node involvement, lymphatic invasion, perineural invasion, deep spread, subsequent treatment with radiation or chemotherapy, and death from disease. Their study included 89 kidney transplant patients, 45 heart transplant patients, 8 lung transplant patients, 5 liver transplant patients, 1 pancreas

transplant patient, and 10 combined transplant patients. They did not find a significant association between type of transplant and any of the factors of aggressiveness.<sup>56</sup>

However, it is possible their ability to detect a significant association was limited by the small sample size in some of the transplant type categories. Adjusting their analyses for significantly longer follow-up in the transplant group, Lott *et al.* still found significantly increased incidence of primary tumors, deep tissue spread, perineural and lymphatic invasion, recurrence, and the need for radiation or chemotherapy in their population of SOTRs, suggesting that SCCs may behave more aggressively post-transplant.<sup>56</sup>

Lott *et al.* identify as a potential confounder in their study the high level of surveillance for skin cancers at their center, suggesting that the true aggressiveness of SCCs in SOTRs may be even greater than what was found. However, a significantly lower number of SCCs in the immunocompetent control patients was captured by their study, and the SOTR group was followed for a significantly longer time overall. If patients in the control group changed providers or became otherwise lost to follow-up in the significantly different follow-up interval, their significantly lower total SCC count and aggressive outcomes could have partially arisen from incomplete capture of events in this retrospective study, complicating the true picture of aggressiveness.

Overall, the evidence appears to substantiate that a subset of SCCs in SOTRs can behave aggressively. Reliable prognostic models that accurately predict risk of recurrence and metastasis based on known risk factors have yet to be developed for SCC or for SCC in SOTRs.<sup>57</sup> As nodal disease, metastasis, and death from aggressive SCC are quite rare outcomes, long-term, prospective, multicenter studies will be needed to identify these risk factors and optimize management. The consensus for clinical practice by transplant providers, based on clinician experience and primarily small retrospective

studies, is to treat all SOTR skin cancers overall as being more likely to recur locally and to metastasize. A significant proportion of SCCs with nodal metastasis can be cured with appropriate therapy, including surgery and adjuvant radiotherapy.<sup>58</sup>

Early definitive diagnosis and treatment are critical to optimizing SCC outcomes. SOTRs should ideally receive care from a dermatologist well-versed in providing SOTR risk-appropriate photo-education, preventative care, and surveillance. Total body skin examinations should be done every three to six months for up to five years following SCC diagnosis, since 95% of local recurrences and metastases are thought to occur within this timeframe.<sup>59</sup> Radiological imaging of the lymph node basin every six months may also be appropriate after diagnosis and treatment of an aggressive SCC.<sup>57</sup> These recommendations will continue to be updated as more data become available.



### **Statement of Purpose**

Solid organ transplant recipients develop up to 100-fold more SCCs compared to the general population, which contributes to substantial morbidity and mortality among this population. SCCs may behave more aggressively in SOTRs versus in immunocompetent patients, though more studies are needed to substantiate this hypothesis. Understanding the clinical course of SCCs in SOTRs, including potential prognostic factors, is crucial to determining proper management of these potentially life-threatening lesions in this high-risk population. This study aims to contribute to a better understanding of the properties of SCCs occurring in a population of SOTRs, which could influence patient screening and counseling, as well as pave the way for future research.

### **Hypothesis**

I hypothesize that solid organ transplant recipients may develop more numerous and more aggressive cutaneous squamous cell carcinomas compared to immunocompetent patients.

### **Specific Aims**

To evaluate patient-level and tumor-level differences between a cohort of solid organ transplant recipients who develop cutaneous squamous cell carcinomas and a cohort of immunocompetent patients who also develop comparable cutaneous squamous cell carcinomas.

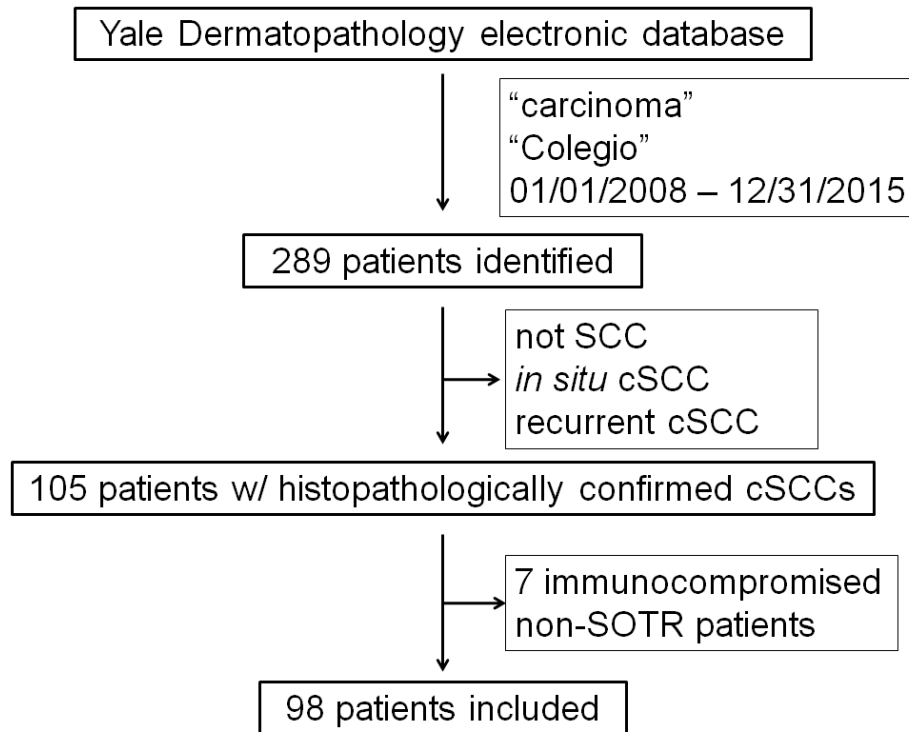
## Methods

Unless otherwise specified, all procedures were performed by the author.

With the approval of the Yale University Institutional Review Board, the complete Yale Dermatopathology electronic database was searched for all carcinoma specimens biopsied by an academic dermatologist specializing in transplant-related cutaneous conditions at a specialized transplant dermatology clinic during the 8-year period between January 1, 2008, and December 31, 2015. Two hundred and eighty-nine patients were identified who had a biopsy that resulted in a pathology report containing the term “carcinoma.”

All of these dermatopathology reports were reviewed, and patients who did not have a diagnosis of primary SCC, e.g. basal cell carcinoma, in situ (noninvasive) SCC, and recurrent SCC, were excluded. The electronic medical records of the 105 patients with at least one histopathologically confirmed SCC were then reviewed to determine their immune status. Seven patients who had compromised immune systems for reasons other than having a solid organ transplant, such as receiving a bone marrow transplant, losing their transplant to rejection before the study period, or taking immunosuppressive medications for other medical conditions like psoriasis or lymphoma, were excluded. Ninety-eight patients who were either immunocompetent or solid organ transplant recipients and had at least one histopathologically confirmed SCC were included in the study (Figure 1).

Figure 1. Study flow chart of patient selection.



A standard database software application (Microsoft Excel, Microsoft Corporation, Redmond, WA) was used to store demographic data collected from the electronic medical record of these patients, including date of birth, gender, race, and immune status (immunocompetent or solid organ transplant recipient). Other variables collected on all patients included date of first visit with a transplant dermatology provider, total number of visits at the transplant dermatology clinic within the study period, date of first biopsy histopathologically confirmed as SCC, and total number of biopsy procedures undergone by the patient within the study period.

Additional data collected on the solid organ transplant patients included date(s) of transplant(s), organ(s) transplanted, number of transplant operations undergone by the

patient, and last confirmed immunosuppressive medications taken by patient within the study period. Patient outcomes through the study period such as local recurrence, regional metastasis, nodal metastasis, disease-specific death, and overall death were also recorded. Patients were included in the study until their death, their last visit with the transplant dermatologist provider, or the study end date, whichever occurred first.

Other calculated patient variables examined included patient age at study end, number of years of total immunosuppression, and total patient follow-up time. Patient age was censored as either age at end of study inclusion, at death, or six months after their last transplant dermatology clinic visit, if they became lost to follow-up. The number of years a transplant patient was on immunosuppressive medications was approximated from the difference between the patient's date of first transplant operation and the patient's final date of inclusion in the study, to the nearest half-year.

A database of every skin biopsy diagnosis received by each patient from the transplant dermatology clinic was then created, and the total numbers of biopsy-confirmed actinic keratoses (AK), non-melanoma skin cancers (NMSC), basal cell carcinomas (BCC), SCCs, and skin tumors overall with which each patient was diagnosed within the study period were determined. From these counts and the individual follow-up time for each patient, an average annual rate of lesion development was calculated.

For each individual SCC tumor from each subject, anatomic site of the tumor, biopsy date, gross description, histologic characteristics such as differentiation and presence of perineural invasion, and margin information was collected from dermatopathology reports. For purposes of analysis, anatomic sites on which the tumors

were diagnosed were sorted into five regions—head and neck (excluding the ear and temple), ear and temple, trunk, upper extremities, and lower extremities. Tumors were assumed to be well-differentiated, unless specifically noted to be moderately or poorly differentiated. Absence of perineural invasion was assumed unless otherwise noted. Margins were assumed to be clear unless otherwise noted on the report.

Data on tumor depth was also collected on a subset of the SCCs in this study. Due to resource limitations on obtaining specimens, a subgroup of SCCs comprised of the first new biopsy-confirmed SCC diagnosed during each patient's follow-up at transplant dermatology clinic, if available, was randomly selected from the total population of SCCs diagnosed in the patient population within the study period. Seventy-seven SCCs, one per patient, were chosen in this manner to control for potential biases in tumor thickness arising from delay in establishing care. Once a patient has established care at the transplant dermatology clinic, the patient is guaranteed an appointment within the week should any dermatologic condition arise, minimizing delay to presentation for new skin lesions. Specimens were re-reviewed by a board-certified dermatopathologist (Dr. Ko), who agreed with histologic diagnosis of SCC in 56 specimens and measured the tumor depth (Figure 2). In cases where the deep margin of the tumor was transected, the tumor was noted to have at least the depth of that measurement. The keratoacanthoma subtype of SCCs were factored as having tumor depth of 0 mm.

Figure 2. Diagnoses upon secondary histopathologic examination of arbitrarily selected specimens initially read as squamous cell carcinoma.

SOTR (n = 45)	Immunocompetent (n = 31)
Histopathologically re-confirmed SCC: 29	Histopathologically re-confirmed SCC: 19
Keratoacanthoma: 4	Keratoacanthoma: 4
Not SCC: 11	Not SCC: 8
Missing: 1	

### ***Protection of patient confidentiality***

In compliance with the requirements of the Institutional Review Board of Yale University, all data was stored in a password-protected Microsoft Excel spreadsheet saved on a Yale network secure server specifically encrypted for the storage of electronic protected health information. Medical record review and data access was conducted only in a private location. Only the investigators listed on the protocol had access to the data.

### ***Statistical analysis***

Demographic data were summarized using mean (standard deviation, range) or median (interquartile range) for continuous variables and frequency (%) for categorical variables. Group comparisons were done using  $\chi^2$  test or Fischer's exact test for categorical variables and t test or Wilcoxon-rank sum test for continuous variables, as appropriate. Age- and gender-adjusted negative binomial regression with robust sandwich estimator was used to account for overdispersion of tumor count data. Follow-up time was used as offset variable to estimate incidence rate. Poisson regression was used to compare margin positivity between groups. The generalized estimating equation (GEE) method was used to compare tumor location between groups while taking correlation

between multiple tumors from the same patient into account.

The significance level was  $p < 0.05$ , two-sided. Data summarization and group comparisons were performed using Stata/SE 13.1 (StataCorp LP, College Station, TX). Negative binomial regression, Poisson regression, and GEE contrast estimates were performed with the assistance of Fang-yong Li, a Yale Center for Analytical Sciences biostatistician, using SAS 9.4 (SAS Institute Inc., Cary, NC).

## Results

### *Patient demographics*

Ninety-eight patients were included in the study (Fig. 1). Of the 98 total subjects, 68 (69.4%) were male, and 30 (30.6%) were female; 96 (98%) were Caucasian, and 2 (2%) were Hispanic/Latino. The average age of the patients was 68.4 years (SD 10.2 years, range 42-91). Demographic data for all subjects is shown in Table 1. There were no significant differences in gender and race distribution between the two groups. However, the immunocompetent group was significantly older than the SOTR group (73.4 vs. 65.0 years,  $p < 0.001$ ).

### *SOTR characteristics*

Some transplant data for the SOTR group is shown in Table 1. Forty-six (79.3%) of the SOTRs underwent one transplant operation, while 12 (20.7%) underwent more than one transplant operation. Of the 46 patients who underwent one transplant operation, 26 were kidney patients, 9 were heart patients, 4 were lung transplant patients, 3 received a liver transplant, and 4 were combined kidney/pancreas transplant patients. The remaining twelve patients underwent between 2 and 4 different transplant operations involving some combination of the previously listed transplanted organs.

A wide range of years of cumulative immunosuppression was represented in this study. The average number of years the SOTRs were immunosuppressed was 14.6 years (SD 9.2, range 2-37). The median number of years of immunosuppression was 12 years (interquartile range 7-20.5). At the end of the study, the most represented immunosuppressive regimen consisted of some combination of mycophenolate, prednisone, and tacrolimus, observed in 10 (17.2%) of 58 patients.



### *Patient care metrics*

A total follow-up of 369 patient-years were included in the study. The 98 subjects were seen at 1,026 office visits at the transplant dermatology clinic over the course of the study period. They underwent 1,866 biopsies of suspicious skin lesions. Data on patient care for all subjects is shown in Table 2. There were no significant differences observed in mean follow-up time (3.8 years vs. 3.7 years,  $p = 0.238$ ), mean number of office visits per patient (11 vs. 9,  $p = 0.819$ ), or mean number of skin biopsies per patient between the two groups (3.8 years vs. 3.7 years,  $p = 0.819$ ). However, after taking variable follow-up time per patient into account, patient visit frequency (number of office visits per patient per year) was significantly higher for the SOTR group compared with the immunocompetent group (4 vs. 3,  $p = 0.025$ ). Annual biopsy rate (number of skin biopsies per patient per year) was also significantly greater in the SOTR group than in the immunocompetent group (6 vs. 5,  $p = 0.039$ ).

### *Tumor counts*

Of the 1,866 skin biopsies performed on the 98 subjects, 640 were histologically diagnosed as AKs, and 756 were diagnosed as NMSCs. One hundred and fifteen of the NMSCs were BCCs. Two hundred and seventy-eight of the NMSCs were SCCs. The remaining NMSCs were essentially SCC *in-situ*. The SCC:BCC ratio of this study population was 2.42:1.

Data on the number of skin lesions diagnosed in each group is shown in Table 3. The SOTR group was diagnosed with a greater number median of AKs than the immunocompetent group (4.5 vs. 2.5). Similarly, the SOTR group was diagnosed with a greater median number of NMSCs overall than the immunocompetent group (5.5 vs. 2.0) and a greater median number of SCCs than the immunocompetent group (2 vs. 1).

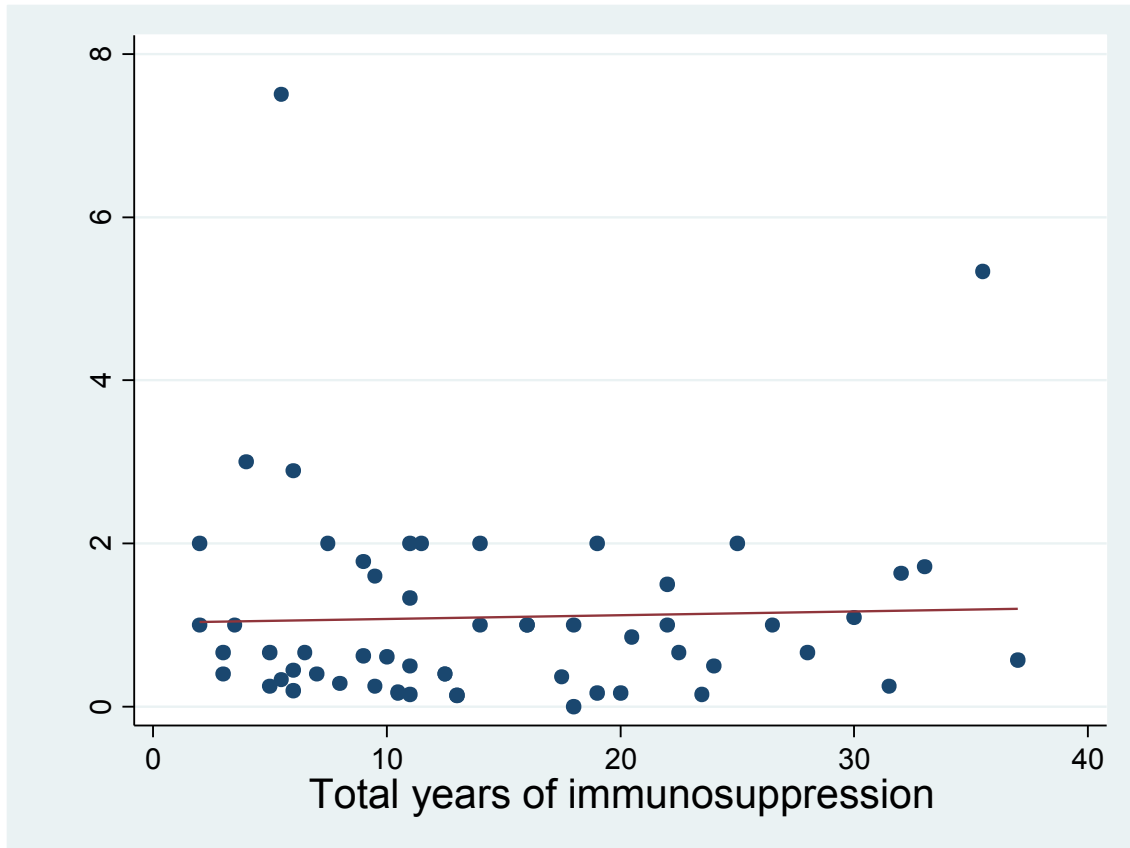
However, no significant differences between the incidence rates of AKs, SCCs, or NMSCs in general were observed between the SOTR and immunocompetent groups. Age and gender were not significant predictors of lesion incidence rates.

*Normalized tumor development rates*

Average annual SCC and NMSC development rates, calculated by dividing the number of SCCs and NMSCs occurring in each patient within the 8-year study period by the patient's variable follow-up time, are shown in Table 3. They were not significantly different between the two groups.

The relationship between average annual SCC development rate and the approximate number of years each patient had been immunosuppressed for transplant at the end of their inclusion in the study was then examined in a scatterplot (Figure 3). There are two clear outlier patients with exceptionally high average annual rates of SCC development compared with the rest of the study population—one patient who recently began immunosuppression and one who had been immunosuppressed for almost thirty years. Excluding these outliers, the average annual rate of SCC development in this cohort of SOTRs does not appear to increase appreciably with increased cumulative years of immunosuppression. Expressed in an alternative way, the average rate of SCC development over the 8-year study period appears to remain constant with increasing years of immunosuppression in this cohort.

Figure 3. Scatterplot of cumulative years of immunosuppression at the end of the study for each SOTR subject (N=58) vs. the annual average rate of SCC development (tumors/year) of each subject over the study period.



### *Outcomes*

Patient outcomes including local recurrence, regional metastasis, nodal metastasis, disease-specific death, and overall death were examined in the study population. No local recurrences were observed among the 98 subjects within the study follow-up period. There were two regional metastases, both occurring in SOTR patients. One 71-year-old male lung transplant recipient was found to have a regional metastasis about eight months after he was diagnosed with the source SCC. An 84-year-old male kidney transplant recipient was also diagnosed with a regional metastasis of a scalp SCC. One occurrence of nodal metastasis was noted in a 74-year-old female kidney transplant patient nine months after she was diagnosed with the likely source SCC. No disease-specific death was observed in the study population. Nine (22.5%) patients in the immunocompetent group and 8 (13.8%) SOTR subjects passed away of non-SCC-related causes during the study period. There was no significant difference in the frequency of death between the SOTR and immunocompetent groups ( $p = 0.263$ ).

### *SCC Tumor Characteristics*

The 98 patients in this study developed 278 histologically confirmed SCCs within the 8-year study period. Of these 278 SCCs, 111 (40%) came from the 40 immunocompetent subjects, while 167 (60%) came from the 58 SOTRs.

### *Perineural invasion*

Of all of the SCCs captured in the study, only one SCC (0.4%) demonstrated evidence of perineural invasion. This SCC was noted to be acantholytic on histology and was located on the scalp of a male heart transplant recipient (1/167; 0.6%).

### *Differentiation*

The majority of SCCs observed in both groups were well-differentiated except for

four SCCs. One male combined kidney/pancreas transplant recipient developed a moderately differentiated SCC on his right cheek, which was measured to be at least 1.65 mm deep. One male kidney transplant recipient developed a poorly differentiated SCC on his left inner cheek, which was measured to be at least 0.55 mm deep. Another male heart transplant recipient developed a poorly differentiated SCC on his right helix, which was measured to be at least 1.1 mm deep. Finally, one female kidney transplant recipient was found to have a metastatic lesion of infiltrative SCC in her right axilla. The metastasis measured 1.6 cm in diameter and was found to be poorly differentiated. She was treated with wide local excision and sentinel lymph node biopsy by plastic surgery. Four of 14 nodes samples were positive, so she also received adjuvant radiotherapy.

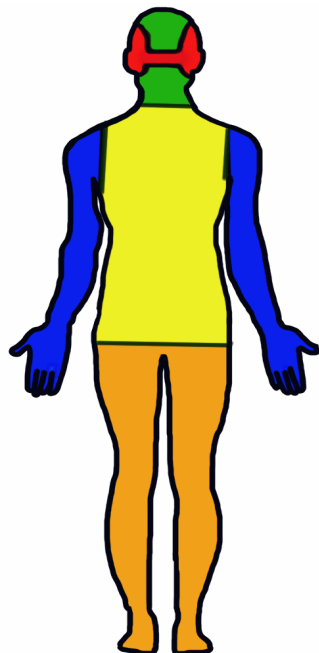
#### *Location*

Tumor locations were grouped into five anatomic regions for analysis, as shown in Figure 4—head and neck excluding the high-risk ear and lip region, the high-risk ear and lip region, the trunk, the upper extremities, and the lower extremities. The number of patients in each group who developed SCCs in each of the anatomic regions is summarized in Table 4, along with the number of SCCs occurring in each region. Thirty-two SOTR patients developed 67 (40.1%) SCCs, while 15 immunocompetent patients developed 24 (21.6%) SCCs in the head and neck region excluding the high-risk zone of the ear and lip. Twelve SOTR patients developed 16 (9.6%) SCCs, while 5 immunocompetent patients developed 8 (7.2%) SCCs in the high-risk zone of the ear and lip. Ten SOTR developed 14 (8.4%) SCCs, while 15 immunocompetent patients developed 21 (18.9%) SCCs on the trunk. Twenty-one SOTRs developed 51 (30.5%) SCCs, while 15 immunocompetent patients developed 27 (24.3%) SCCs on the upper extremities. Ten SOTRs developed 19 (11.4%) SCCs, while 12 immunocompetent

patients developed 31 (27.9%) SCCs on the lower extremities.

There were no significant differences in risk of developing an SCC observed in the ear and lip region, on the upper extremities, or on the lower extremities between the SOTR and immunocompetent groups (Table 4). However, the risk of developing an SCC in the head and neck region was 90% greater in the SOTR group than in the immunocompetent group (RR=1.89, 95% CI 1.04-1.37). In addition, the difference in risk of developing an SCC in the trunk region trended toward significance between the two groups—the risk appeared to be approximately half in the SOTR group compared with in the immunocompetent group (RR=0.46, 95% CI 0.21-1.03).

Figure 4. Body map showing the five anatomic regions into which SCC tumor locations were sorted—head and neck excluding high-risk ear and lip region (green), high-risk ear and lip region (red), trunk (yellow), upper extremities (blue), and lower extremities (orange).



*Margin positivity*

Margins were clear in 225 (81%) of the 278 SCCs biopsied in the study population, while positive margins were noted in 53 (19%) of the SCCs biopsied. Data on biopsy margin status is summarized in Table 5. Seventeen (15.3%) of the SCCs biopsied in immunocompetent patients were found to have positive margins, while 36 (21.6%) of the SCCs biopsied in SOTRs were found to have positive margins. No significant difference in the risk of positive margins on biopsy was observed between the immunocompetent and SOTR groups.

*Tumor depth*

Tumor depth was measured by a board-certified dermatopathologist in 56 arbitrarily selected histologically confirmed SCC specimens from the study cohort—specifically, the first available sample for each patient once follow-up at transplant dermatology clinic had been established. The average number of years SOTR subjects had been immunosuppressed at the time their SCC was included in this analysis was 12.4 years (SD 9.1, range 0.5-34.5). The median years of immunosuppression was 10.5 (IQR 5-18.5). The overall mean tumor depth measured in all specimens was 1.24 mm (SD 0.43 mm, range 0-2.5 mm). The median tumor depth measured among all specimens was 1.20 mm (IQR 0.9-1.55). The median tumor depth measured in tumors from 33 SOTR patients was 1.15 mm, while the median tumor depth measured in tumors from 23 immunocompetent patients was 1.05 mm. Data on tumor depth is summarized in Table 6. There was no significant difference in tumor depth noted between the two groups in this study population.

Table 1. Baseline properties of the study population.

		SOTR (N = 58)			Immunocompetent (N = 40)			<i>p</i>	
	n (%)	Mean±SD	Median	IQR	n (%)	Mean±SD	Median	IQR	
<b>Demographics</b>									
Age, years		65.0±8.7	65.5	59-71		73.4±10.3	73.5	52-91	< 0.001 <sup>a</sup>
<b>Gender</b>									
Male	44 (75.9)				24 (60.0)				0.094
Female	14 (24.1)				16 (40.0)				
<b>Race</b>									
White	56 (96.5)				40 (100.0)				0.235
Hispanic or Latino	2 (3.5)								
<b>Transplant Data</b>									
<b>No. of Transplants</b>									
1	46 (79.3)								
>1	12 (20.7)								
Immunosuppression, years		14.6±9.2	12	7-20.5					
SOTR: solid organ transplant recipient; SD: standard deviation; IQR: interquartile range. <sup>a</sup> Highlights significant association.									



Table 2. Comparison of patient care received by the study population.

	SOTR (N = 58)		Immunocompetent (N = 40)		p
	Mean±SD	Median (IQR)	Mean±SD	Median (IQR)	
Follow-up time, years	3.8±2.2	4 (1.5-6)	3.7±2.4	3.75 (1-6)	0.819
No. office visits	11±8	11 (5-16)	9±9	7 (3-15)	0.238
Visit frequency	4±2	3 (2-4)	3±2	2 (2-3)	0.025 <sup>a</sup>
No. skin biopsies	21±17	14 (8-34)	17±17	11 (4-24)	0.247
Annual biopsy rate	6±4	6 (3-8)	5±3	4 (2-7)	0.039 <sup>a</sup>

SOTR: solid organ transplant recipient; SD: standard deviation; IQR: interquartile range. Visit frequency and annual biopsy rate calculated using variable follow-up time for each patient. <sup>a</sup> Highlights significant association.

Table 3. Comparison of tumor incidence in the study population.

	SOTR (N = 58)		Immunocompetent (N = 40)		IRR (95% CI)	p
	Mean±SD	Median (IQR)	Mean±SD	Median (IQR)		
No. AKs	7±8	4.5 (1-10)	6±9	2.5 (0.5-5)	SOTR: 1.84 (1.37, 2.48) IC: 1.32 (0.91, 1.93)	1.39 (0.85, 2.27) 0.17
No. NMSCs	8±8	5.5 (3-11)	7±8	2 (2-13)	SOTR: 2.55 (2.03, 3.20) IC: 2.20 (1.59, 3.05)	1.16 (0.79, 1.69) 0.50
No. of SCCs	3±3	2 (1-4)	3±4	1 (1-3)	SOTR: 1.03 (0.74, 1.43) IC: 0.87 (0.63, 1.20)	1.19 (0.79, 1.79) 0.54
Annual SCC rate	1.1±1.3	0.67 (0.3-1.6)	1.2±1.2	0.62 (0.3-2)	--	-- 0.97
Annual NMSC rate	2.6±2.5	2 (1-3)	2.2±2.1	1.9 (0.7-3.6)	--	-- 0.38

Annual lesion rate calculated as an average of number of lesions captured within study period over variable follow-up time for each patient. IR: incidence rate; IRR: incidence rate ratio (SOTR vs. immunocompetent); CI: confidence interval; AK: actinic keratosis; NMSC: non-melanoma skin cancer; SCC: squamous cell carcinoma.

Table 4. Comparison of anatomic location of tumors in the study population.

	<b>SOTR</b> <b>n (%), N</b>	<b>Immunocompetent</b> <b>n (%), N</b>	<b>RR</b>	<b>95% CI</b>	<b>p</b>
Head and neck <sup>a</sup>	67 (40.1%), 32	24 (21.6%), 15	1.90	1.04-3.47	0.038 <sup>b</sup>
Ear and lip	16 (9.6%), 12	8 (7.2%), 5	1.38	0.42-4.56	0.598
Trunk	14 (8.4%), 10	21 (18.9%), 15	0.46	0.21-1.03	0.059
Upper extremities	51 (30.5%), 21	27 (24.3%), 15	1.28	0.57-2.86	0.552
Lower extremities	19 (11.4%), 10	31 (27.9%), 12	0.42	0.14-1.24	0.118

<sup>a</sup> except high-risk ear and lip region. <sup>b</sup> Highlights a significant association. n = number of SCCs (% total SCCs in group); N = number of patients; RR: relative risk (SOTR vs. immunocompetent); CI: confidence interval.

Table 5. Comparison of tumor margin positivity in the study population.

	<b>SOTR</b> <b>n (%), N</b>	<b>Immunocompetent</b> <b>n (%), N</b>	<b>RR</b>	<b>95% CI</b>	<b>p</b>
Margins positive	36 (21.6%), 5	17 (15.3%), 15	1.39	(0.78, 2.5)	0.27
Margins clear	131 (78.4%), 12	94 (84.7%), 32			

n = number of SCCs (% total SCCs in group); N = number of patients. RR: relative risk (immunocompetent vs. SOTR).

Table 6. Comparison of tumor depth in a subset of SCCs from study population.

	<b>SOTR</b> <b>(N = 33)</b>		<b>Immunocompetent</b> <b>(N = 23)</b>		<b>p</b>		
	<b>Mean ±SD</b>	<b>Median</b>	<b>IQR</b>	<b>Mean ±SD</b>	<b>Median</b>	<b>IQR</b>	
Depth, mm	1.11±0.61	1.15	0.08-1.55	0.98±0.57	1.05	0.65-1.55	0.261

## Discussion

The ~100-fold increased incidence of SCC in SOTRs compared with the general population is well-documented in the literature.<sup>22-25</sup> Varied small retrospective studies with disparate definitions of aggressiveness have led to the belief that SCCs in SOTRs generally behave more aggressively than those in the general population.<sup>42,48</sup> A recent larger-scale study comparing SCCs in SOTRs with those in immunocompetent patients supports this observation of statistically more aggressive behavior by SCCs in SOTRs.<sup>56</sup> The consensus for clinical practice by transplant providers is therefore to treat all SOTR skin cancers as being more likely to recur locally and to metastasize. The purpose of this study was to gather more data regarding differences in behavior between SCCs in iatrogenically immunosuppressed SOTRs and those occurring in a stringent control group of immunocompetent patients over an 8-year study period.

Based on a number of measures, the SCCs developed by our SOTR group did not appear to behave more aggressively than those developed by our immunocompetent control population. One measure of aggressive behavior of SCCs used in this study is poor outcomes at the patient level. Poor outcomes in SCC are generally difficult to compare statistically because of their rarity, so we described them in our study. There were no incidents of local recurrence or of disease-specific death in either group over the 8-year study period. One incident of perineural invasion was noted in the SCC of a heart transplant patient. Regional metastasis occurred in a lung transplant and a kidney transplant patient, and nodal metastasis was noted in one kidney transplant recipient. Though all poor outcomes in this study occurred in the SOTR group, there were too few to determine if the differences are statistically significant.

Several studies have proposed that certain histologic features of SCCs, such as

acantholytic and infiltrative characteristics, may be associated with a more aggressive clinical course, and that SOTRs appear to develop thicker, more infiltrative SCCs.<sup>47,60</sup> Brantsch *et al.* assessed 615 immunocompetent white patients over a median follow-up period of 43 months and found that SCCs greater than 2.0 mm in thickness showed a significant tendency to metastasize. In addition, they found that tumors greater than 6.0 mm in thickness were associated with a high risk of metastasis and local recurrence.<sup>61</sup> Of 56 arbitrarily selected histologically-confirmed SCC specimens from our study cohort, chosen as the first available sample for each patient once follow-up at transplant dermatology clinic had been established, the thickest SCC and the only SCC greater than 2.0 mm in thickness in the overall cohort was 2.5 mm thick and occurred in an SOTR. However, we did not find a significant difference in tumor depth between the two groups in our study population. That is, we did not find the SCCs of the SOTR group to be significantly thicker than those of the immunocompetent group. The average number of years our SOTR subjects had been immunosuppressed at the time their SCC was biopsied was over a decade, and there was no significant difference in the risk of positive margins upon biopsy of SCC between the two groups, meaning that biopsy behavior and technique were not influenced by the patient's immune status. We reported degree of differentiation since it is often reported as a grading criterion in SCC, but it does not appear to have significant prognostic value.

The primary difference we detected between SCCs occurring in our SOTR group compared with the SCCs occurring in our immunocompetent control group was tumor location. We observed a 90% greater risk of developing SCC in the head and neck region in the SOTR group compared with the immunocompetent group (RR=1.89, 95% CI 1.04-1.37). The difference in risk of developing an SCC in the trunk region trended toward

significance between the two groups—SOTRs had approximately half the risk of developing trunk SCCs compared with in the immunocompetent group (RR=0.46, 95% CI 0.21-1.03). The increased risk of developing SCCs on the head and neck in SOTRs may reflect their increased susceptibility to UV-induced carcinomas, especially if they are taking calcineurin inhibitors that disable UV-damage repair mechanisms as part of their immunosuppressive regimen. The trend toward decreased risk of developing SCCs on the trunk of SOTRs is possibly a statistical anomaly but interesting. Both of these trends could represent differences in anatomical site distribution of SCCs due to factors like age and sex that were not directly analyzed for this study. Lindelof *et al.* examined anatomical site distribution of 475 SCCs in 179 SOTRs and noticed differences in distribution based on age and sex. In particular, they found that male SOTRs tended to develop SCCs on their head and neck, while female SOTRs predominantly developed SCCs on their trunk; in addition, younger patients tended to develop SCCs on their chest, while older patients developed more SCCs on their face.<sup>62</sup> A recent study of cutaneous lower extremity SCCs suggested that regional anatomic variation in cutaneous susceptibility could arise from varying numbers of cutaneous dendritic cells among body sites.<sup>63</sup> Perhaps iatrogenic immunosuppression shifts cutaneous immunity in the truncal region. This trend could also represent the SOTRs in the study population being particularly well-educated about wearing sunscreen, being more vigilant about sun exposure, and wearing protective clothing covering their trunk compared to their immunocompetent counterparts.

The immunosuppressive medications SOTRs take for the entirety of their graft function are thought to drive the drastically increased risk of SCCs in SOTRs compared with immunocompetent patients, and perhaps the increased aggressive behavior.<sup>35,36</sup> In

comparing SCC outcomes between our SOTR study group and our control study immunocompetent group, it was therefore important to ensure that the immunocompetent control group was comparable to the SOTR group in as many meaningful ways as possible, aside from the iatrogenic immunosuppression of the SOTRs. Our SOTR group was demographically comparable with our immunocompetent control group with regards to gender and race distribution. The SOTR group was younger as a whole compared with the immunocompetent control group, and this is consistent with the well-documented earlier onset of SCCs in SOTRs in the literature; SOTRs typically begin developing SCCs 3 to 5 years after transplant.<sup>42,43,56</sup> Therefore, age was not matched between groups to obtain a better representation of the true differences between the groups. Our SOTR and immunocompetent groups were of comparable health status in that there was no significant difference in the frequency of death between groups.

Though our SOTR group was diagnosed with a greater absolute median number of AKs, NMSCs, and SCCs than the immunocompetent group overall, the differences observed between the incidence rates of AKs, SCCs, or NMSCs between the SOTR and immunocompetent groups were not statistically significant; the SOTR group developed a statistically comparable number of lesions with our immunocompetent control group. In addition, the average annual development rate of either SCCs or NMSCs was also comparable between the two groups. This contrasts with a previous study comparing SCCs occurring in a large population of SOTRs with a significantly smaller number of SCCs in a matched population of immunocompetent patients.<sup>56</sup>

An important factor that could limit a study's ability to detect the true differences between groups is the amount of medical care received by patients; for example, Lott *et al.* statistically controlled for variable follow-up time in their large-scale study, but it is

possible that outcomes of interest might have gone uncaptured by their study as follow-up time was significantly less among the immunocompetent patients.<sup>56</sup> Our study examined differences in patient care provided to the two groups by comparing follow-up time, number of office visits per patient, and number of skin biopsies per patient for each group. Overall, we did not find significant differences between our two study groups in any of these measures. Adjusting for variable follow-up time per patient, our SOTR group did have significantly greater patient visit frequency (4 office visits per patient per year *vs.* 3,  $p = 0.025$ ) and significantly greater annual biopsy rate (6 skin biopsies per patient per year *vs.* 5,  $p = 0.039$ ) compared with our immunocompetent group. The greater annual visit frequency and biopsy rate reflect strict adherence to current dermatologic surveillance recommendations for SOTRs and greater clinical suspicion about the clinical course of the lesions they develop.

A wide range of years of cumulative immunosuppression was represented among the SOTR subjects in the study. A weakly positive correlation between average annual SCC development rate and years of cumulative immunosuppression per patient was observed, though it was driven by two clear outliers—one patient with a very high rate of SCC development after many years of immunosuppression and one with a very high rate of SCC development shortly after starting immunosuppression. Intrinsic genetic factors or other as of yet not well-understood characteristics may predispose these outlier patients to extreme rates of annual SCC development. Excluding these outlier patients, the rate of SCC development does not appear to increase with increasing cumulative years of immunosuppression. That is, immunosuppressive medications appear to pose a fixed, rather than increasing, risk of carcinogenesis over time in our SOTR study group. This is a surprising observation given numerous studies showing that the heightened risk



of skin cancers increases with greater cumulative time on immunomodulatory therapy and with increasing intensity of the regimen.<sup>35</sup> Though chemoprevention of SCCs in SOTRS was not directly examined in this study, its availability to SOTRs receiving care at this clinic could potentially account for the largely fixed annual SCC development rate among SOTRs with varying cumulative years of immunosuppression.

In sum, our study compared SCCs developed by a cohort of SOTRs over 8 years with SCCs developed by a stringent control cohort of demographically comparable non-immunosuppressed individuals who received comparable patient care and who developed comparable numbers of skin lesions. In this highly-controlled setting, we did not find the SCCs developed by SOTRs to be significantly more aggressive than those in our immunocompetent control group. Nor did we appreciate an increased risk of carcinogenesis over time in our SOTR group.

While interesting, the results of this study should be interpreted carefully in the context of the strengths and limitations of this study. Our detection of rare aggressive outcomes in SCC was limited by the relatively small sample size of the study. A considerable 369 patient-years of follow-up were included in this 8-year retrospective study, but larger, multi-center studies would be needed to compare rare poor outcomes in SCCs among SOTRs. As such, we were limited to describing the poor outcomes that occurred in our cohort.

Another weakness of this study was that it was conducted at a single institution, which limits its generalizability to populations dissimilar from that served by the Yale-New Haven Transplantation Center. However, this could also be considered a strength because it controls for variations in biopsy technique, which we showed to be comparable between our two groups by examining rate of margin positivity, and for hospital-related

confounders.

Identification of subjects and SCCs for inclusion in the study relied upon clinical pathology reports, which are somewhat subjective. A board-certified dermatopathologist collaborator reviewed all specimens included in the study, but having a panel of dermatopathologists to ensure intra- and inter-observer reproducibility of specimen interpretation would have been ideal.

Another potential weakness of this non-randomized retrospective observational cohort study is that it was conducted in a high-risk referral population with timely access to specialized transplant care, which unfortunately limits its generalizability to all SOTR patients in general. The immunocompetent control group was also composed of high-risk skin cancer patients who were referred to a tertiary center. While this may introduce referral bias, it actually strengthens comparisons between our SOTR and immunocompetent patients in that the control immunocompetent group stringently matched the SOTR group with regards to possible confounders such as demographic composition, patient care received, and general tumor outcomes.

The primary difference between our two groups besides immunosuppression of the SOTR group was significantly higher annual visit frequency and annual biopsy rate among the SOTRs. Point estimate differences tell us that SOTRs in this clinic presented for care roughly every 3 months, while their high-risk immunocompetent counterparts presented every 4 months on average. In addition, the SOTRs developed lesions suspicious enough to warrant biopsy roughly 6 times a year, while their high-risk immunocompetent counterparts had lesions biopsied roughly 5 times a year. Though the differences were statistically significant, the absolute difference is marginal—1 visit and 1 biopsy per year.

Taken together, our findings suggest that the drastically increased risk of SCCs in SOTRs and the potentially more aggressive clinical course of SCCs may be successfully reduced to a level comparable to that in high-risk immunocompetent individuals with close adherence to current dermatologic surveillance recommendations for SOTRs and a marginally lower threshold for biopsy of suspicious lesions.

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