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# Tacrolimus Levels Are Not Associated with Risk of Malignancy in Lung Transplant Recipients

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Data Collection B  
Statistical Analysis C  
Data Interpretation D  
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**Background:** Lung transplant (LTx) recipients suffer from high rates of malignancy. Exposure to immunosuppressive medication such as tacrolimus has been proposed as a risk factor for tumorigenesis. We hypothesized that chronically high levels of tacrolimus would be associated with risk of malignancy.

**Material/Methods:** The study was performed in a transplant center in Israel, with a nested case-control design. Cases were LTx recipients who were diagnosed with any solid or hematological malignancy except non-melanoma skin cancer. Controls were tumor-free during their entire follow-up after LTx and had at least the same follow-up time as their matched case. Controls were matched to cases by age and type of transplant received (single/double). Tacrolimus levels were extracted and analyzed for median drug level and also integrated over time (area under the curve – AUC-tacrolimus).

**Results:** We reviewed 412 LTx recipients in our registry. Thirty-nine cases of malignancy were diagnosed and 160 controls were matched, giving a crude tumor incidence rate of 26/100 000/year. Lung cancers were the commonest diagnosis. Cases and controls were well matched by age, smoking status, and LTx type. Median tacrolimus levels were 11.0 ng/ml and 11.3 ng/ml in cases and controls, respectively ( $p=0.88$ ). The median log (AUC-tacrolimus) was 9.4 in the cases and 9.5 in the controls ( $p=0.59$ ).

**Conclusions:** In this nested case-control study, exposure to tacrolimus was similar in tumor cases and non-tumor controls. These data, based on a cohort with modest size, suggest either that tumorigenesis in LTx recipients is unrelated to tacrolimus exposure or that levels in these patients are above an unknown threshold at which the dose-response effect is saturated.

**MeSH Keywords:** **Carcinogenesis • Case-Control Studies • Immunosuppressive Agents • Lung Transplantation • Neoplasms • Tacrolimus**

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## Background

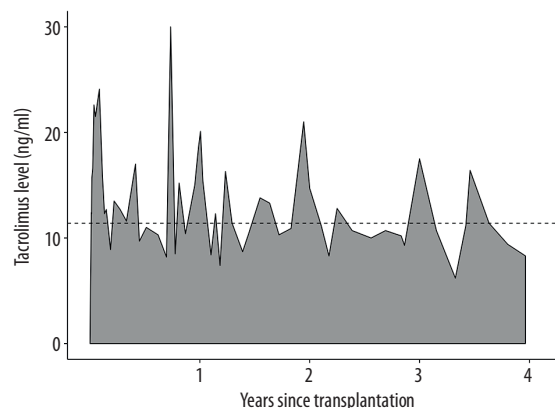
While lung transplantation (LTx) is the gold standard treatment for end-stage lung diseases, the long-term survival of recipients still lags behind that of other solid-organ recipients. Malignancies make up a significant portion of post-transplant morbidity and mortality in LTx recipients [1].

Several explanations may be offered as to why LTx recipients have high rates of cancers [2]. LTx recipients are typically middle-aged adults, and many have been prior smokers. The latter fact is particularly important when single LTx is performed, since the retained native lung was exposed to high levels of carcinogens. Indeed, many past case series have described high rates of native lung bronchial carcinoma [3–8]. Immunosuppressive medication could conceivably allow proliferation of tumorigenic viral infections such as Epstein-Barr virus (associated with post-transplant lymphoproliferative disease) and Human Herpes-8 virus (Kaposi sarcoma) and Cytomegalovirus. Another plausible explanation is that the immunosuppressive medication itself may reduce the anti-tumor effects of the immune system and allow tumorigenesis to progress unhindered [2,9]. Previous studies have also associated the use of voriconazole with risk of developing skin carcinoma [10].

The aim of the present study was to determine the incidence of tumors in our LTx recipient database and whether exposure to immunosuppressive medications, specifically chronically high levels of tacrolimus, is associated the risk of malignancy.

## Material and Methods

The study was a retrospective nested case-control study and was approved by the Institutional Research Ethics Committee. Rabin Medical Center's lung transplant program is the only national program in Israel. In our program, active malignancy within 5 years of referral is a contraindication for listing. In addition, all potential LTx candidates are screened for occult malignancy by CT scan during pre-transplantation work-up. We reviewed the files of all lung transplant recipients from 1998 to the end of 2012, and searched for biopsy results indicating malignancy. Potential cases/controls were excluded if they were treated with cyclosporin or were transferred to mTOR inhibitors during follow-up. The standard protocol for immunosuppression following LTx in our center is tacrolimus (tac), mycophenolate, and prednisolone. There is no routine use of induction therapy. Tac levels are adjusted to 12–20 ng/ml immediately postoperatively to 8–12ng/ml during long-term follow-up. Recipients were defined as cases if any solid-organ or hematological malignancy was diagnosed during the post-transplantation period, with the exception of non-melanoma skin cancer. Each potential case was evaluated independently



**Figure 1.** Representative sample showing tacrolimus levels over time in a single patient from the moment of transplantation (time zero) until tumor diagnosis. The dashed line represents median level. The shaded area is the AUC-tac measurement.

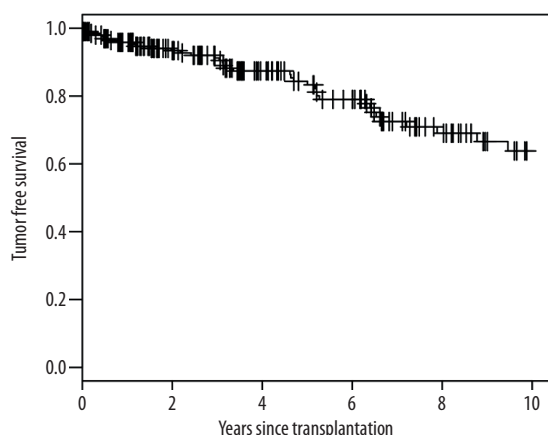
by 2 investigators. Controls were matched by age at transplantation, single-double lung transplant status, and had survival time at least as long as the case to which they were matched. We attempted to match 3–5 controls to each case, and any 1 individual could not be included as a control more than once. Matching of cases to controls was by computer algorithm.

### Tacrolimus exposure

Data on tacrolimus (tac) levels were queried from the hospital laboratory computer system. For each subject, we created a time-series of data points (time vs. tac level) from the date of transplantation until the censoring date (example, Figure 1). Median tac level was calculated for each subject. To model total exposure to tac, we integrated tac levels over time (area under the curve [AUC]) using the trapezoid rule, which we defined as 'tac-AUC'. In control patients, we truncated the time-series data at time of tumor-free survival of their matched case.

### Statistical analysis

Descriptive data are summarized as median (inter-quartile range [IQR]) or as counts. The groups were compared by univariate analysis with the Wilcoxon test or chi-square/Fisher's test, as appropriate. A multivariate analysis of between-group factors was planned based on the results of the univariate analysis. Tac-AUC data were log-transformed prior to analysis (logAUC). All tests were two-tailed and the significance level was set at  $p < 0.05$ . A Kaplan-Meier curve of tumor-free survival was generated (Figure 2). We used R (version 3.1.1) for all analysis (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>).



**Figure 2.** Kaplan-Meier curve of tumor-free survival in the cohort of cases and matched controls.

## Results

We identified 39 tumor cases and matched 160 controls among the overall 412 LTx recipients in our database (Table 1). The total tumor-free survival time in the case-control cohort was 920.4 person years, yielding a crude tumor incidence of 26/100 000/year. The case and controls were well matched by age (56–59 years) and LTx type, as planned. Without prior matching, there were no differences between the groups in terms of sex (63–64% male), diagnosis, and prior smoking status (74–78%). In the tumor cases, median survival after tumor diagnosis was 35 weeks (IQR 5-101). The distribution of malignancies is shown in Table 2. Lung cancers dominated the diagnoses, with 21 cases diagnosed. Of note, 16 cases of lung cancer were diagnosed in the native lung of single LTx recipients ( $p=0.048$ ), of whom 13 were past cigarette smokers.

**Table 2.** Breakdown of malignancy cases diagnosed in LTx recipients.

Tumor site	Cases	Details
Lung	21	Squamous 8
		Adenocarcinoma 5
		Undifferentiated 3
		Small cell 2
		Bronchoalveolar 2
		Clear 1
		Native lung 16
		Transplanted lung 5
PTLD	6	
Gastrointestinal	5	Colon 3
		Stomach 1
		Cholangiocarcinoma 1
Urogenital	4	Bladder TCC 3
		Renal cell 1
Breast	2	
Thyroid	1	

The cases and controls were well matched by follow-up time and number of tac levels available per case (Tables 1, 3). Median tac levels were 11.0 ng/ml and 11.3 ng/ml in cases and controls, respectively ( $p=0.88$ ). In the tac-AUC analysis median logAUC was 9.4 in the cases and 9.5 in the controls ( $p=0.59$ ). Given

**Table 1.** Demographic data of cases and matched controls. Data presented as median (interquartile range) or counts/percents. The case vs. control groups are compared with Wilcoxon test or chi square, as appropriate ( $p$ -value).

	Cases	Controls	p
Number in group	39	160	
Age (y)	56 (53–63)	59 (53–62)	0.59
Sex (Male%)	64	63	0.99
Prior smoking (% yes)	78	74	1.00
Single lung transplant (%)	85	87	0.91
Baseline diagnosis			0.73
Emphysema	36	41	
Fibrosis	46	39	
Other	18	20	
Tumor Free Survival (weeks)	175 (71–332)	187 (86–376)	0.35

**Table 3.** Analysis of Tacrolimus levels. Data presented as median (inter-quartile range). P value calculated with the Wilcoxon test.

	Cases	Controls	p
Median (ng/ml)	10.9 (10.0–12.0)	11.3 (9.9–12.2)	0.88
Log(tacrolimus-AUC)	9.4 (9.0–10.0)	9.5 (8.6–10.0)	0.59
Measurements	53 (30–72)	52 (26–74)	0.88

that there were no between-group variables that differentiated between the tumor cases and the controls in all the univariate comparisons, we did not perform a multivariate analysis.

## Discussion

We performed a case-control study of LTx recipients to test the hypothesis that chronic high-level exposure to tacrolimus is associated with risk of tumorigenesis. We showed that tac exposure was not a quantitative risk factor for tumors. The only risk factor in the cohort was single-lung transplantation. The largest subgroup of patients were ex-smokers receiving single LTx and who developed lung cancer in the native lung.

While the excess incidence of malignancies in solid-organ transplant recipients is well described, the underlying causative mechanism are not clearly understood [3]. Intuitively, immunosuppressive medication is an attractive explanatory factor because immune surveillance has been shown to be important in prevention of malignancy and immunosuppressive medication may encourage viral tumorigenesis [2]. Consequently, it would be expected that a dose-response relationship would exist between intensity of immunosuppression (either tac levels or more individual medications) and tumor diagnosis. Our study is novel in that we estimated the total exposure for each patient based on laboratory measurements of tac levels, both as a simple intensity (median level) and also in the tac-AUC, which integrates time of exposure with intensity of exposure. We conclude that tacrolimus exposure alone cannot explain the excess malignancy rate in the LTx recipients. These results are also consistent with good-quality evidence from other sources. In a meta-analysis, tacrolimus was not different from cyclosporin in terms of tumorigenesis [11]. Registry data also failed to show increased tumor risk associated with tac therapy, and even suggested a decreased risk associated with addition of mycophenolate [12]. Furthermore, in a randomized trial of tacrolimus vs. tacrolimus-mycophenolate immunosuppression in heart transplant recipients, reports of tumors were not increased in the latter, more intensely immunosuppressed group, although the study was not powered or intended to measure this outcome [13]. In contrast, studies in liver and kidney transplant recipients found a dose-effect relationship [14,15]. Overall, our data and other reports

are inconsistent between different recipient populations and, specifically in LTx, the data do not support the hypothesis that more intense immunosuppression is a significant risk factor for tumorigenesis.

Our study has a number of strengths in addition to the novelty of the analytical technique. By using a carefully matched case-control design, we were able to isolate confounders such as age and LTx type (single vs. double). In addition, each control was analyzed at the same time point as their matched case, enabling us to eliminate bias resulting from longer survival in the controls. The main weakness in our study is the modest sample size of the cancer cases, although previous single-center studies reporting malignancies in LTx patients had similar-sized cohorts, and the screened cohort was large (412 cases). It is possible that there is a type I statistical error, but given that the p values were far from the level of significance, we believe that the null hypothesis is in fact correct. In addition, it is possible that there is a 'threshold' level of tacrolimus required for tumorigenesis, which is well below the typical levels in our cohort, but without a dose-response effect above that threshold. In studies of liver and kidney recipients who were less intensely immunosuppressed than our cohort, a dose-effect was found between tacrolimus exposure and subsequent malignancy [14,15]. We also urge other centers to replicate our study, and we are willing to collaborate with other centers by sharing the computer algorithm for extracting and analyzing the laboratory data. A second limitation is that we did not assess non-melanoma skin cancers in the study. Such cancers are frequently treated by local dermatologists and plastic surgeons rather than in our center; therefore, the data could not easily be obtained.

Perhaps the most important finding in the present study was the alarming excess of lung cancer cases occurring in the native lung among prior smokers, accounting for 13/39 cancer cases. The survival advantage of double LTx has been clearly demonstrated in multiple studies, and our study suggests that some of the survival benefit may be accounted for by eliminating the risk of native lung carcinoma. When the transplant team decides whether to transplant 2 recipients with a single lung or one with both allografts, we believe that the risk of native lung carcinoma should be taken into consideration, along with other factors.

## Conclusions

In a large cohort of 412 LTx recipients, exposure to chronically high tacrolimus levels was not associated with carcinogenesis in LTx; rather, the main risk factor was receipt of a single-lung allograft.

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