Journal of Surgical Research **171,** 1–5 (2011)
doi:10.1016/j.jss.2011.03.068

ASSOCIATION FOR ACADEMIC SURGERY

Increased Levels of Tumor-Infiltrating Lymphocytes are Associated with Improved Recurrence-Free Survival in Stage 1A Non-Small-Cell Lung Cancer

Zachary D. Horne, B.S.,*,[1] Robert Jack, B.S.,* Zachary T. Gray, B.S.,* Jill M. Siegfried, Ph.D.,† David O. Wilson, M.D.,‡ Samuel A. Yousem, M.D.,§ Katie S. Nason, M.D., M.P.H.,* Rodney J. Landreneau, M.D.,* James D. Luketich, M.D., F.A.C.S.,* and Matthew J. Schuchert, M.D.*

*Division of Thoracic Surgery, Heart, Lung, and Esophageal Surgery Institute, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania; †Department of Pharmacology, Lung and Thoracic Malignancy Program, University of Pittsburgh Cancer Institute, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania; ‡Division of Pulmonary Allergy and Critical Care Medicine, Department of Medicine, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania; and §Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania

Originally submitted January 8, 2011; accepted for publication March 25, 2011

Background. **Tumor-infiltrating lymphocytes (TILs) have been found to increase survival in many forms of cancer, including, endometrial, bile ductal, colonic, esophageal, and urothelial cancers, as well as melanoma and follicular lymphoma. The relevance of TILs in the prognosis of non-small-cell lung cancer (NSCLC), however, still remains controversial. We compared the outcomes of stage 1A NSCLC with and without tumor infiltrating lymphocytes to evaluate the effects of TILs on recurrence and survival patterns.**

Materials and Methods. **From 2000 to 2009, 273 anatomic segmentectomies and lobectomies were performed on stage 1A NSCLC. Patients were stratified into TIL– and TIL+ cohorts based on pathologic evaluation. Further investigation was conducted on the effects of TILs in patients with and without angiolymphatic invasion. Variables analyzed include overall survival, recurrence-free survival, and type of recurrence.**

Results. **Overall 5-y survival was not affected by TIL status (65% *versus* 60%, $P = 0.469$). Five-year recurrence-free survival (RFS) was significantly increased in the TIL+ group *versus* the TIL– group (87% *versus* 73%, $P = 0.011$), most significantly in women ($P = 0.016$). The presence of angiolymphatic invasion (ALI) was associated with decreased 5-y RFS *versus* patients without ALI (61% *versus* 85%, $P < 0.001$). Interestingly, in the ALI negative group, TIL+ patients experienced a significantly increased 5-y recurrence-free survival *versus* TIL– patients (93% *versus* 80%, $P = 0.036$).**

Conclusions. **High levels of intratumoral TILs are associated with improved recurrence-free survival in stage 1A NSCLC patients as well as a reduced likelihood of systemic recurrence. When angiolymphatic invasion is not present, the beneficial effects of TILs become even more profound.** Published by Elsevier Inc.

Key Words: **thoracic surgery; non-small-cell lung cancer; angiolymphatic invasion; tumor infiltrating lymphocytes.**

INTRODUCTION

Complete surgical resection continues to be the primary therapeutic approach for early-stage tumors. Lobectomy has long been considered the gold standard for the treatment of stage 1A non-small-cell lung cancer (NSCLC), however it has been shown that anatomic segmentectomy may be a comparable procedure for low-stage tumors [1]. In spite of this, locoregional and systemic recurrences occur in 10% to 30% of pathological stage 1A patients 5 y post-resection[2–4]. The host immune response may play a role in reducing recurrence rates, as a vigorous immune response may be a marker of better prognosis.

Tumor-infiltrating lymphocytes, especially CD8+ cytotoxic T-lymphocytes, have been found to increase survival in many forms of cancer, including NSCLC, endometrial, bile ductal, colonic, esophageal, and

[1] To whom correspondence and reprint requests should be addressed at Division of Thoracic Surgery, Heart, Lung, and Esophageal Surgery Institute University of Pittsburgh Medical Center, Shadyside Medical Building, 5200 Centre Avenue, Pittsburgh, PA 15232. E-mail: hornezd@gmail.com.

0022-4804/$36.00
Published by Elsevier Inc.

urothelial cancers, as well as melanoma and follicular lymphoma [5–12]. The survival conferred by tumor-infiltrating lymphocytes (TILs) in NSCLC, however, has been subject to much controversy. Pelletier *et al.* found peritumoral B- but not T-cells to be an indicator of better survival [12]. Hiraoka *et al.* found that both CD8+ and CD4+ T–cells improve survival only when tumoral invasion was concurrent [13]. Nakamura *et al.* found absolutely no prognostic significance for intratumoral T-cells [14]. Peterson *et al.* and Chen *et al.* found that the only statistically significant lymphocyte was the CD25+ regulatory T-cell (T-reg) cell, which indicated a greater likelihood of recurrence [15, 16].

Further muddying the waters, Kawai *et al.*, Carrega *et al.*, Al-Shibli *et al*, and Zhuang *et al.* have all demonstrated that survival depends not only on the TIL makeup, but also the cellular distribution patterns [17–20]. Their various conclusions indicate that CD8+ cells in the cancer nest and stroma are useful biomarkers for predicting prognosis, NK cells within cancer nests are likely unable to kill tumor cells yet maintain an active cytokine environment, and high densities of CD4+ cells in the stroma are positively correlated with outcomes. Ruffini *et al.* found similar and additional distributional significances, but perhaps more importantly, found that TIL status was only significant to prognoses in squamous cell cancer [21].

The multitude of studies on the significance of TILs to the prognosis of cancer seems to show only that there is little consensus on the effects of TILs to the outcome of NSCLC. Conclusive data are limited and little is known about the variable effects of TIL population cell types and spatial distribution in resected early-stage tumors. Therefore, based upon findings in the literature and our institutional observations, we have hypothesized that in resected stage 1A NSCLC with formal pathologic evaluation, the presence of tumor-infiltrating lymphocytes will have a positive impact on recurrence-free survival.

MATERIALS AND METHODS

Patients

Approval for this study was provided by the Institutional Review Board of the University of Pittsburgh and patient consent was waived. We performed a retrospective review of 273 patients with pathologic stage 1A NSCLC who underwent anatomical segmentectomy or lobectomy *via* open thoracotomy or video-assisted thoracoscopic surgery between 2000 and 2009 at the University of Pittsburgh Medical Centers. Patients were identified from the billing records of the Heart, Lung, and Esophageal Surgery Institute and patient chart review.

As a part of normal pathologic workup at the University of Pittsburgh Medical Center, resected NSCLC is evaluated for tumor infiltrating lymphocytes by the Department of Pathology and is classified as having low, moderate, or high infiltration of lymphocytes. Low infiltration indicates scattered lymphocytes within the stroma; high infiltration denotes an intense stromal lymphocytic presence as well as lymphocytes percolating between tumor cells, while moderate infiltration includes only a modest stromal lymphocytic presence without tumor nest permeation. Patients with low infiltration (TIL–) were compared against those with moderate and high infiltration (TIL+). Primary outcomes included overall survival, recurrence-free survival, and type of recurrence (locoregional *versus* distant/systemic).

Statistical Analysis

Comparison of the TIL– and TIL+ cohorts was performed using clinical and pathologic data. Survivals were defined as the time from surgery to the date of death or recurrence, or in the absence of the former, date of last follow-up. The probability of overall and recurrence-free survival was estimated using the Kaplan-Meier method, with significance being assessed by the log-rank test. To compare the frequencies of locoregional *versus* distant or systemic recurrence, as well as the occurrence of increased inflammation within each subpopulation, two-tailed Fisher exact tests were implemented.

RESULTS AND DISCUSSION

Results

The patient demographics and the frequency with which increased TILs were found in each subpopulation can be found in Table 1. Patient and tumor survival statistics are summarized in Table 2 with *P* values representing the significance of 5-y recurrence-free survival in each cohort. Mean age and gender distribution were similar in the negative and positive TIL groups. Average tumor size was 1.9 cm and was similar in both cohorts. The major histologic subtypes were adenocarcinoma and squamous cell carcinoma.

While the presence of tumor-infiltrating lymphocytes had a great impact on the recurrence-free survival of

TABLE 1

Population Demographics and Frequency of TILs

	TIL+	TIL–	*P*
Gender			
Male	48	82	0.067
Female	69	74	
Procedure			
Lobectomy	70	91	0.901
Segmentectomy	47	65	
Histology			
Adenocarcinoma	61	86	0.954
Squamous	41	53	
Large Cell	7	10	
Differentiation			
Poor	42	36	0.0054
Moderate	65	87	
Well	8	26	
Tumor Size			
$\leq$ 2 cm	74	104	0.608
2–3 cm	43	52	
Angiolymphatic invasion			
ALI–	83	113	0.788
ALI+	34	43	