ORIGINAL ARTICLE

Obesity-Specific Association of Statin Use and Reduced Risk of Recurrence of Early Stage NSCLC

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ABSTRACT

Introduction: Statins, used for their lipid-lowering activity, have anti-inflammatory and anticancer properties as well. We evaluated this potential benefit of statin use in patients with NSCLC.

Methods: All 613 patients with pathologic stage 1 or 2 NSCLC who had lobectomy without neoadjuvant therapy at our institution during 2008 to 2015 were included. Association between presurgery statin use and overall survival and recurrence-free survival (RFS) was analyzed using Cox proportional hazards regression. Association of statin use with tumor transcriptome was evaluated in another 350 lung cancer cases.

Results: Univariable analyses did not reveal a statistically significant association of statin use with either overall survival or RFS, with hazard ratio equals to 1.19 and 0.70 (Wald $p = 0.28$ and 0.09), respectively. In subgroup analyses, significantly improved RFS was found in statin users, but only in overweight/obese patients (body mass index [BMI] > 25; n $= 422$), with univariable and multivariable hazard ratio of 0.49 and 0.46 ($p = 0.005$ and 0.002), respectively, but not in patients with BMI less than or equal to 25 (n $= 191$; univariable $p = 0.21$). Transcriptomes of tumor statin users had high expression of tumoricidal genes such as granzyme A and interferon-γ compared with those of nonusers among high- but not low-BMI patients with lung cancer.

Conclusions: Our study suggests that statins may improve the outcome of early stage NSCLC but only in overweight or obese patients. This benefit may stem from a favorable reprogramming of the antitumor immune response that statins perpetrate specifically in the obese.

Keywords: Body mass index; Lung cancer; Obesity; Statin; Tumor immunity

Introduction

Lung cancer is a major cause of cancer-related deaths in the United States and worldwide. As with many other human cancers, prolonged inflammation is thought to promote the development and progression of lung cancer.[1] Although such inflammation is induced by tobacco smoking, other factors also contribute to systemic inflammation, obesity perhaps being the most important one worldwide. There were 38% and 41% of the adult male and female populations, respectively, in the United States who were obese during 2015 to 2016,[2] with body mass index (BMI) greater than or equal to 30 kg/m^2. A substantial fraction of cancers of at least eight solid

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tissues is associated with being overweight or obese.[3] In step with the increasing incidence of obesity, rates of these obesity-associated cancers in the United States increased by 7% during 2005 to 2014.[4] Although lung cancer has not been generally regarded as obesity associated, consideration of obesity may be important for patients with lung cancer, up to two-thirds of whom are overweight or obese in the United States.[5]

The statin class of lipid-lowering drugs is used for their ability to inhibit the mevalonate pathway to reduce the production of cholesterol and other isoprenoids. Statins are in wide use, with 28% of U.S. adults aging more than 40 years estimated to have received them in 2013.[6] In 2015, the American Diabetes Association expanded its statin therapy recommendation to include all those with diabetes of 40 to 75 years in age. The widespread use of statins has facilitated the identification of potential secondary, noncardiovascular benefits of these drugs, such as reduced risks of pancreatitis, contrast-induced nephropathy,[7] recurrence, and cancer-specific mortality for a variety of solid tumors, including lung cancer.[8,9] In a large case-control study of U.S. Veterans, Khurana et al.[10] revealed that statin use is associated with a decreased risk of lung cancer. This observation was also made in a large population-based study from Taiwan that focused on female patients.[11] Similarly, a large retrospective study of the U.K. population revealed that statin use before the diagnosis of lung cancer was associated with reduced disease-specific mortality.[12] Nevertheless, as elegantly revealed by Suissa et al.,[13] studies such as these may be susceptible to false-positive results owing to time-window bias. Indeed, many studies have failed to reveal any association of statin use with cancer incidence or survival[13,14] or have found adverse association with cancer incidence.[15] Importantly, a robust meta-analysis of well-designed and adequately powered randomized clinical trials of statins in cancer also failed to confirm efficacy of statins for solid malignancies.[16]

It is possible that these conflicting results and the uncertainty surrounding the anticancer benefit of statins arise from variations in patient characteristics, such as the underlying condition that indicated statin therapy. Obesity may be another confounder, as suggested by our recent study revealing the survival benefit of using the antidiabetic drug metformin in NSCLC may be restricted to only those who are overweight or obese.[17] In this study, analysis of immune-relevant genes in the tumor microenvironment suggested that despite the well-established immune dysfunction expected in obese animals and individuals,[18,19] metformin use was associated with favorable reprogramming of antitumor immunity unique in patients with lung cancer with high BMI.[17] Similar to that of metformin, the anticancer benefit of statins may be particularly potent under the chronic proinflammatory conditions that exist in obesity. It is known that statins can directly affect lung cancer cells to reduce cell proliferation, epithelial-mesenchymal transition, and migration.[20–22] This is presumably driven by the modulation of pathways that involve proteins, such as EGFR, Ras enzymes, and transforming growth factor-β1. Biological effects of statins that are anti-inflammatory in nature and independent of their lipid-lowering action have also been established with both clinical trials and experiments with cells in vitro and in the mouse.[23–25] Given these known anti-inflammatory effects of statins and the uniquely dysregulated immune landscape in the obese, we hypothesized that if indeed statins are able to improve lung cancer outcomes, obesity-specific immune modulation may be responsible. The goal of our present study was thus to evaluate the impact of obesity on association of statin use with survival in NSCLC, using a uniform cohort of patients with similar stage and treatment.

Materials and Methods

Ethical Statement

This retrospective study was approved by the institutional review board of Roswell Park Comprehensive Cancer Center (RPCCC), Buffalo, New York. Requirement for informed consent of the study subjects was waived because the study required only a retrospective review of medical records.

Clinical Data of Early Stage NSCLC Cohort

Institutional general thoracic surgery database and cancer registries were accessed to retrospectively identify all patients with a diagnosis of pathologic stage 1 or 2 primary NSCLC who underwent lobectomy without neoadjuvant therapy at RPCCC between 2008 and 2015. This time period was chosen to allow for an adequate follow-up duration. More than 90% of patients had their surgery performed by video-assisted thoracoscopic surgery. Routine lymph node dissection or sampling was performed for all patients. Information on age, history of tobacco smoking (current, former, and never categories), and BMI at time of diagnosis, gender, race of patients, stage (as per the seventh edition of the staging manual of the American Joint Committee on Cancer), histologic grade (low, $\leq$2, and high, $\geq$3), and histology (adenocarcinoma, squamous cell carcinoma, and other NSCLC categories) was collected from patient medical records. Data for forced expiratory volume at 1 second as percentage of predicted ($FEV1_{pred}$), American Society of Anesthesiologists (ASA) physical status class (low, $\leq$2, and high, $\geq$3) at time of surgery, cancer recurrence, and survival after surgery were also

collected. Overall survival (OS) was defined as the time from surgery to last follow-up or death. Recurrence-free survival (RFS) was the time from surgery to diagnosis of recurrence or last follow-up or death if recurrence was not diagnosed. Statin exposure data were compiled from multiple sources, including prescription histories, inpatient and outpatient medication administration records, medication verifications performed by institute staff, and inpatient and outpatient prescription charges in pharmacy and financial records. If statin use was documented with any of these means, the patient was considered a statin user. This was based on the logic that a medication use may be missed, but is rarely falsely included. An audit of 50 randomly selected patients of this study revealed that all patients who were deemed as statin users before surgery were also using the drug after surgery at 6 months or the first surveillance visit if it was after 6 months. Nevertheless, it is still an assumption for remaining patients. To avoid time-window bias,[13] only patients who were receiving statins at the time of surgery were considered exposed to statins. As statins are generally prescribed long term and not episodically, patients prescribed statins before surgery were assumed to continue them afterward. Survival and NSCLC recurrence data were abstracted from the institutional cancer registry, which uses histologic confirmation or documentation by the treating physician in the absence of the former to determine recurrence. Differentiation between recurrence and second primary was as per the guidelines of the United States Surveillance, Epidemiology, and End Results program.

Targeted Transcriptome Profiling of NSCLC Tumors

For 350 patients with lung cancer treated at RPCCC, tumor transcriptome data were obtained by OmniSeq (Buffalo, NY) using U.S. Clinical Laboratory Improvement Amendments–licensed platforms for therapy guidance as part of clinical care. Hematoxylin-eosin–stained sections of formalin-fixed, paraffin-embedded tumor tissue blocks were evaluated by a board-certified anatomical pathologist to ensure greater than or equal to 5% cellular content and less than or equal to 50% necrosis. RNA extracted from the formalin-fixed, paraffin-embedded samples was subjected to targeted sequencing that covered 397 cancer- and immunity-related genes as previously described.[26] Gene expression was derived from the sequencing data after background subtraction, normalization against a set of housekeeping genes, and conversion to percentile ranking with respect to a reference set of 735 solid tumors of 35 histologies.[26]

Statistical Analyses

All analyses were performed using R (version 3.6.2). For two-group comparisons, standard t test, Fisher's exact test, and Wilcoxon ranked sum tests were respectively used for continuous, categorical, and ordinal variables, respectively. For survival analyses, the survival R package (version 3.1-12) was used. For multivariable Cox regression modeling, all covariates with likelihood ratio test p value less than or equal to 0.10 in univariable Cox regression models were included, and the assumption of proportional hazards was tested with Schoenfeld residuals using the package's cox.zph function. The assumption of linearity of continuous covariates was confirmed by inspecting plots of Martingale residuals. Prism (version 8.3.1 for Mac OS; GraphPad Software, San Diego, CA) was used for graphing and log-rank tests for survival analyses. Unless noted otherwise, default values were used for statistical software options, tests were two-tailed, and the threshold of 0.05 was used to deem significance.

Data Availability

The data generated in this study are available on request from the corresponding author.

Results

Statin Use Does Not Associate With Improved Outcomes in Early NSCLC

We evaluated the association of statin use with NSCLC outcomes in a cohort of 613 patients with pathologic stage 1 or 2 NSCLC who were treated by lobectomy without neoadjuvant therapy at RPCCC between 2008 and 2015. Table 1 summarizes important demographic and clinicopathologic variables of this cohort. There were 127 subjects of the cohort (37 with stage 1 and 90 with stage 2 disease) who received adjuvant chemotherapy, and 99 were diagnosed with having recurrence after lobectomy (33 locoregional, 63 distant, and three unknown recurrence sites). Among the 613 patients of the cohort, 261 (35%) were receiving statins at the time of surgery. Compared with the 352 patients who were not taking statins (Table 1), statin users were older (mean ± SD = 69.7 ± 8.9 y versus 65.6 ± 10.5 y, $p < 0.01$) and more likely to be male (48% versus 38%, $p = 0.02$), and had higher BMI (29.1 ± 5.7 versus 26.7 ± 5.6, $p < 0.01$) and ASA physical status class (65% with score ≥3 versus 41%, $p < 0.01$). Although statin users had a greater proportion of nonsquamous cancers (43% versus 36%, $p < 0.01$) and a lower proportion of current smokers (17% versus 32%, $p < 0.01$), the two groups of patients did not significantly differ for race, $FEV1_{pred}$, or tumor stage or histologic grade.

Table 1. Characteristics of Patients of the Study

Characteristic	All (N = 613)	Statin User (n = 261)	Not Statin User (n = 352)	p^a
Age (y) at diagnosis, mean; SD	67.4; 10.1	69.7; 8.9	65.6; 10.5	4.72E−07
Gender, n (%)				1.66E−02
Female	356 (58)	137 (52)	219 (62)	
Male	257 (42)	124 (48)	133 (38)	
Race, n (%)				2.82E−01
Pure Caucasian	502 (82)	219 (85)	283 (81)	
Other	107 (18)	40 (15)	67 (19)	
Tobacco smoking, n (%)				<2.2E−16
Current	155 (25)	44 (17)	111 (32)	
Past	392 (64)	193 (74)	199 (57)	
Never	63 (11)	23 (9)	40 (11)	
BMI, mean; SD	27.8; 5.7	29.1; 5.7	26.8; 5.6	2.86E−07
BMI > 25, n (%)				2.04E−01
No	191 (31)	52 (20)	139 (39)	
Yes	422 (69)	209 (80)	213 (61)	
$FEV1_{pred}$, n; mean; SD	583; 80.9; 20.6	247; 80.6; 22.9	336; 81.1; 22.5	7.78E−01
ASA physical status class, n (%)				9.20E−09
≤2	299 (49)	92 (35)	207 (59)	
≥3	314 (51)	169 (65)	145 (41)	
Tumor histology, n (%)				<2.2E−16
Adenocarcinoma	376 (61)	150 (57)	226 (64)	
Squamous cell carcinoma	170 (28)	78 (30)	92 (26)	
Other	67 (11)	33 (13)	34 (10)	
Tumor pathologic stage (AJCC seventh edition), n (%)				7.83E−01
1	448 (73)	189 (72)	259 (74)	
2	165 (27)	72 (28)	93 (26)	
Tumor histologic grade, n (%)				8.51E−01
≤2	403 (66)	182 (70)	221 (63)	
≥3	210 (34)	79 (30)	131 (37)	
Postsurgery month of follow-up, median; IQR	31.4; 33.8	30.3; 28.7	32.9; 37.4	1.65E−01
Postsurgery month to recurrence, n; median; IQR	99; 13.2; 16.0	34; 14.4; 12.1	65; 13.0; 16.8	5.95E−01
Postsurgery month to death, n; median; IQR	159; 23.8; 24.3	71; 24.6, 23.8	88; 21.9; 25.0	7.36E−01

[a]In comparison of statin user and not statin user groups using 2-tailed standard *t* test and Fisher's exact test for continuous and categorical variables, respectively.

Note: ASA class, BMI, $FEV1_{pred}$, smoking history, and history of statin use are before tumor resection surgery.

AJCC, American Joint Committee on Cancer; ASA, American Society of Anesthesiologists; BMI, body mass index; $FEV1_{pred}$, forced expiration volume at 1 second (% of predicted); IQR, interquartile range.

Univariable analyses using Cox proportional hazards regression revealed the expected significant associations (likelihood ratio test $p < 0.05$) with worse OS of increasing age, male gender (versus female), history of tobacco smoking (former versus current), high tumor grade (≥3 versus other), squamous histology (versus adenocarcinoma), and higher ASA physical status class (≥3 versus other) (Fig. 1 and Table in Supplementary Data 1). In a multivariable Cox model that included these six covariates, the associations with OS remained significant (Wald $p < 0.05$) for all the covariates except smoking history and histology. Similar expected associations of gender, grade, and histology with RFS as for OS was observed in both univariable and multivariable analyses (Fig. 1 and Table in Supplementary Data 1). Statin use was not significantly associated with OS in univariable analysis (Wald $p = 0.28$) or with RFS in either univariable or multivariable analysis ($p = 0.09$ and 0.07, respectively).

Statin Use Is Associated With Reduced Risk of Recurrence of Early NSCLC Only in High-BMI Patients

We also investigated the association of statin use with outcomes separately in the 422 overweight/obese (BMI > 25) and the remaining 191 (BMI ≤ 25) patients of the cohort. In the high-BMI patient group, which had 173 and 16 subjects with BMI greater than 30 and greater than 40, respectively, 209 patients (49.5%) were statin users. Table 2 summarizes the survival analyses performed for this subgroup.

Statin use was not associated with OS in univariable analysis ($p = 0.51$; Fig. 1) and was therefore not included in the multivariable analysis. Nevertheless,

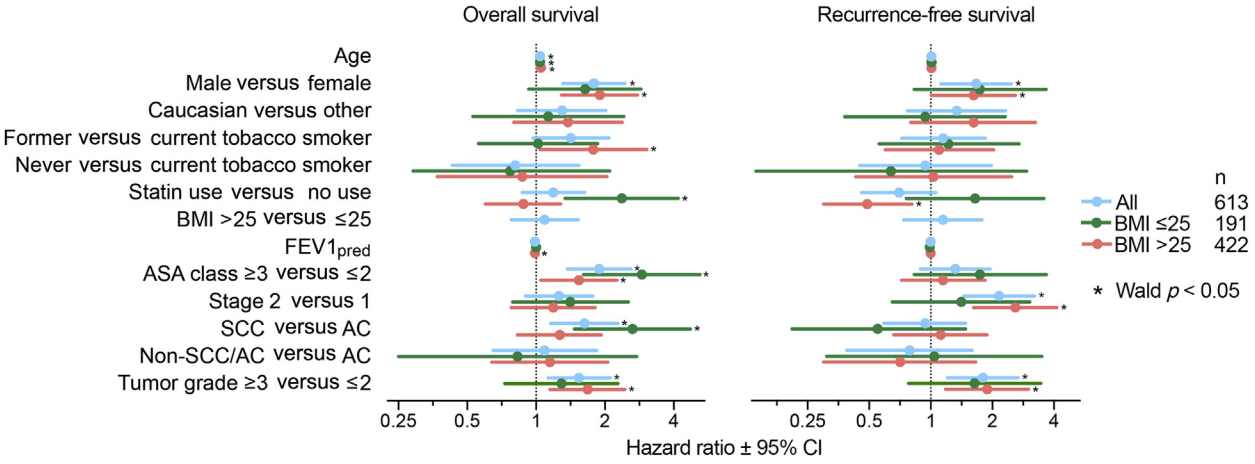

Figure 1. Association of demographic and clinicopathologic variables with survival after lobectomy for early stage NSCLC. HR with 95% CIs for overall and recurrence-free survival in univariable Cox proportional hazards regression models are plotted for all 613 patients with pathologic stage 1 or 2 and their subgroups of 422 obese/overweight subjects with BMI greater than 25 and 191 subjects with BMI less than or equal to 25. Asterisks indicate HR values with Wald *p* value less than 0.05. ASA class, BMI, $FEV1_{pred}$, tobacco smoking history, and history of statin use are before surgery. Pathologic stages are as per the American Joint Committee on Cancer staging manual (seventh edition). AC, adenocarcinoma; ASA, American Society of Anesthesiologists (physical status class); BMI, body mass index; CI, confidence interval; $FEV1_{pred}$, forced expiration volume at 1 second (% of predicted); HR, hazard ratio; SCC, squamous cell carcinoma.

statin use was associated with improved RFS in the high-BMI patients in both univariable and multivariable analyses, with hazard ratio (HR) of 0.49 and 0.46 ($p = 0.005$ and 0.002; Fig. 1), respectively. In a multivariable analysis of RFS that included metformin use as an additional covariate, the significant association of statin use with RFS remained (HR = 0.48, $p = 0.005$; Fig. in Supplementary Data 2) whereas there was no significant association with RFS of metformin use ($p = 0.17$). Evaluation of the 191 patients with BMI less than or equal to 25, 13 of whom had BMI less than or equal to 18, revealed an association of statin use with decreased OS in univariable analysis (HR = 2.37, $p = 0.004$), which, however, was not significant in multivariable modeling (HR = 1.58, $p = 0.14$) (Fig. 1, and Table in Supplementary Data 3). There was no significant association of statin use in these patients with RFS either (univariable HR = 1.65, $p = 0.21$).

The relationships observed between survival (OS and RFS) and various demographic and clinicopathologic covariates in the full study cohort and its two BMI-based subgroups in the univariable Cox models are summarized in Figure 1. Kaplan-Meier curves for OS and RFS among statin users and those not using statin in the three patient groups are found in Figure 2. In obese patients (BMI > 30), statin use was not associated with OS or RFS (univariable $p = 0.24$ and 0.76, respectively). Among overweight but not obese patients (30 ≥ BMI > 25), statin use was associated with RFS (HR = 0.42, $p = 0.009$) but not OS ($p = 0.39$). In a step-wise multivariable Cox analysis of RFS that had the BMI covariate as a continuous variable and included the interaction term for BMI and statin use (BMI*statin), the interaction term but neither BMI nor statin use was retained in the model, further suggesting the effect of statin use on RFS is BMI dependent.

Transcriptomes Suggest BMI-Specific Immunomodulatory Effects of Statins on Lung Cancer Tumors

Having observed the BMI-specific association of statin use with cancer outcome, we sought to assess its biological basis by evaluating lung cancer tumor transcriptomes. As we did not have such data for greater than 90% of our cohort of 613 patients, we chose to use a targeted tumor gene expression data set that had been generated at our institution using Clinical Laboratory Improvement Amendments–approved molecular testing platforms to guide the treatment of 350 patients of lung cancer (235 adenocarcinoma, five large cell carcinoma, 10 small cell carcinoma, 57 squamous cell carcinoma, and 43 not specified NSCLC or lung cancer) of all clinical stages (15 stage 1 or 2, 255 stage 3 or 4, and 80 not specified). Among the 350 patients, at the time of cancer diagnosis, BMI was less than 25 for 153 and greater than or equal to 25 for 197, and 57 and 121 of them, respectively, were taking statins. To avoid confounding by known effects of metformin on tumor gene expression,[17] we excluded metformin users when identifying this cohort of 350 cases.

Analysis of the patients' tumor expression data for the 397 targeted cancer- and immunity-related genes

Table 2. Survival Analyses of Patients With Body Mass Index Greater Than 25 (n = 422) Using Cox Proportional Hazards Regression Models

	Univariable				Multivariable[a]		
Covariate	LRT *p*	Compared groups	Wald *p*	HR (95% CI)	PHA *p*	Wald *p*	HR (95% CI)
Overall survival							
Age	3.74E−05		6.61E−05	1.05 (1.02-1.07)	4.99E−01	2.46E−03	1.04 (1.01-1.07)
Gender	9.26E−04	Male vs. female	1.01E−03	1.90 (1.29-2.78)	1.43E−01	7.98E−02	1.43 (0.96-2.14)
Race	2.30E−01	Caucasian vs. other	2.48E−01	1.38 (0.80-2.38)			
Smoking history	1.67E−02	Former vs. current	3.43E−02	1.78 (1.04-3.05)	3.62E−01	1.70E−01	1.48 (0.84-2.61)
		Never vs. current	7.57E−01	0.87 (0.37-2.04)		9.98E−01	1.00 (0.41-2.42)
ASA class ≥ 3	2.44E−02	Yes vs. no	2.58E−02	1.54 (1.05-2.26)	5.22E−01	4.59E−01	1.17 (0.77-1.76)
$FEV1_{pred}$	2.04E−02		2.00E−02	0.99 (0.98-1.00)	8.06E−01	3.12E−02	0.99 (0.98-1.00)
Statin use	5.07E−01	Yes vs. no	5.07E−01	0.88 (0.60-1.28)			
Tumor grade ≥ 3	7.88E−03	Yes vs. no	7.01E−03	1.68 (1.15-2.45)	4.60E−02	4.57E−02	1.49 (1.01-2.21)
Tumor histology	5.36E−01	SCC vs. AC	2.68E−01	1.27 (0.83-1.93)			
		Other vs. AC	6.34E−01	1.15 (0.64-2.06)			
Tumor stage	4.24E−01	2 vs. 1	4.18E−01	1.19 (0.78-1.81)			
Recurrence-free survival							
Age	5.22E−01		5.24E−01	1.01 (0.98-1.03)			
Gender	4.28E−02	Male vs. female	4.35E−02	1.62 (1.01-2.59)	4.77E−01	5.84E−02	1.59 (0.98-2.55)
Race	1.54E−01	Caucasian vs. other	1.79E−01	1.62 (0.80-3.25)			
Smoking history	9.45E−01	Former vs. current	7.56E−01	1.10 (0.60-2.03)			
	9.45E−01	Never vs. current	9.43E−01	1.03 (0.43-2.49)			
ASA class ≥ 3	5.48E−01	Yes vs. no	5.49E−01	1.15 (0.72-1.84)			
$FEV1_{pred}$	7.73E−01		7.73E−01	1.00 (0.99-1.01)			
Statin use	3.65E−03	Yes vs. no	4.74E−03	0.49 (0.30-0.81)	7.23E−01	2.31E−03	0.46 (0.28-0.76)
Tumor grade ≥ 3	9.11E−03	Yes vs. no	7.91E−03	1.89 (1.18-3.01)	4.10E−03	1.44E−02	1.80 (1.12-2.89)
Tumor histology	5.91E−01	SCC vs. AC	6.79E−01	1.12 (0.66-1.88)			
	5.91E−01	Other vs. AC	4.26E−01	0.71 (0.30-1.66)			
Tumor stage	1.18E−04	2 vs. 1	7.04E−05	2.59 (1.62-4.13)	2.16E−01	6.18E−05	2.63 (1.64-4.21)

Note: Models in Cox multivariable analyses include only covariates with LRT *p* value less than 0.10 in univariable analyses. Tumor pathologic staging is as per the American Joint Committee on Cancer manual, seventh edition. Statin use is for presurgery period. Age and tobacco smoking history are at time of diagnosis. ASA class and $FEV1_{pred}$ are before surgery.

[a]*LRT p* values for the full models are for 7.47E−06 and 7.33E−07 for overall and recurrence-free survival, respectively.

AC, adenocarcinoma; ASA, American Society of Anesthesiologists (physical status); CI, confidence interval; $FEV1_{pred}$, forced expiration volume at 1 second (% of predicted); HR, hazard ratio; LRT, likelihood ratio test; PHA, proportional hazards assumption for covariate (test using Schoenfeld residuals); SCC, squamous cell carcinoma.

revealed that statin use was associated with differential expression (Wilcoxon ranked sum test $p < 0.05$) of 25 genes among the high-BMI patients but only eight genes among the low-BMI patients (table in Supplementary Data 4). With Hochberg's adjustment for multiple testing to reduce false discovery, three (*CCL4*, *GNLY*, and *GZMA*) and one (*CEACAM8*) of the genes, respectively, had adjusted *p* value less than 0.05 (all with higher expression in statin users; Fig. 2). Genes that were overexpressed among statin users compared with nonusers in the high-BMI group included proinflammatory genes associated with tumoricidal immune response—*CD69*, *CD8*, granulysin (*GNLY*), granzyme A (*GZMA*), interferon γ (*IFNG*), perforin 1 (*PRF1*), and *STAT1* and *CTLA4* and *LAG3* immune checkpoint genes. In the low-BMI group, none of these genes was differentially expressed between statin users and nonusers. In contrast, genes associated with myeloid (*LST1*),[27] neutrophil (*FCGR3B/CD16b*),[28] and myeloid-derived suppressor cell lineages (*CEACAM8/CD66b*, *FCER1G*, and *FCGR3B/CD16b*)[29–31] were up-regulated in the low-BMI statin users. These results suggest that statin use can yield a number of BMI-specific immunomodulatory effects that may explain the apparent clinical benefits of the drugs in high-BMI patients with NSCLC. Association of statin use with lung cancer tumor gene expression in patient groups with low and high BMIs is illustrated in Figure 3.

Discussion

It may be possible to counteract the proinflammatory nature of obesity that is promotive of cancer[32] with medications that have anti-inflammatory effects. This was also suggested by our recent work that identified a survival benefit of metformin use only among overweight and obese patients with NSCLC with BMI greater than or equal to 25.[17] In this study, we observe a similar but hitherto unknown phenomenon for statin use.

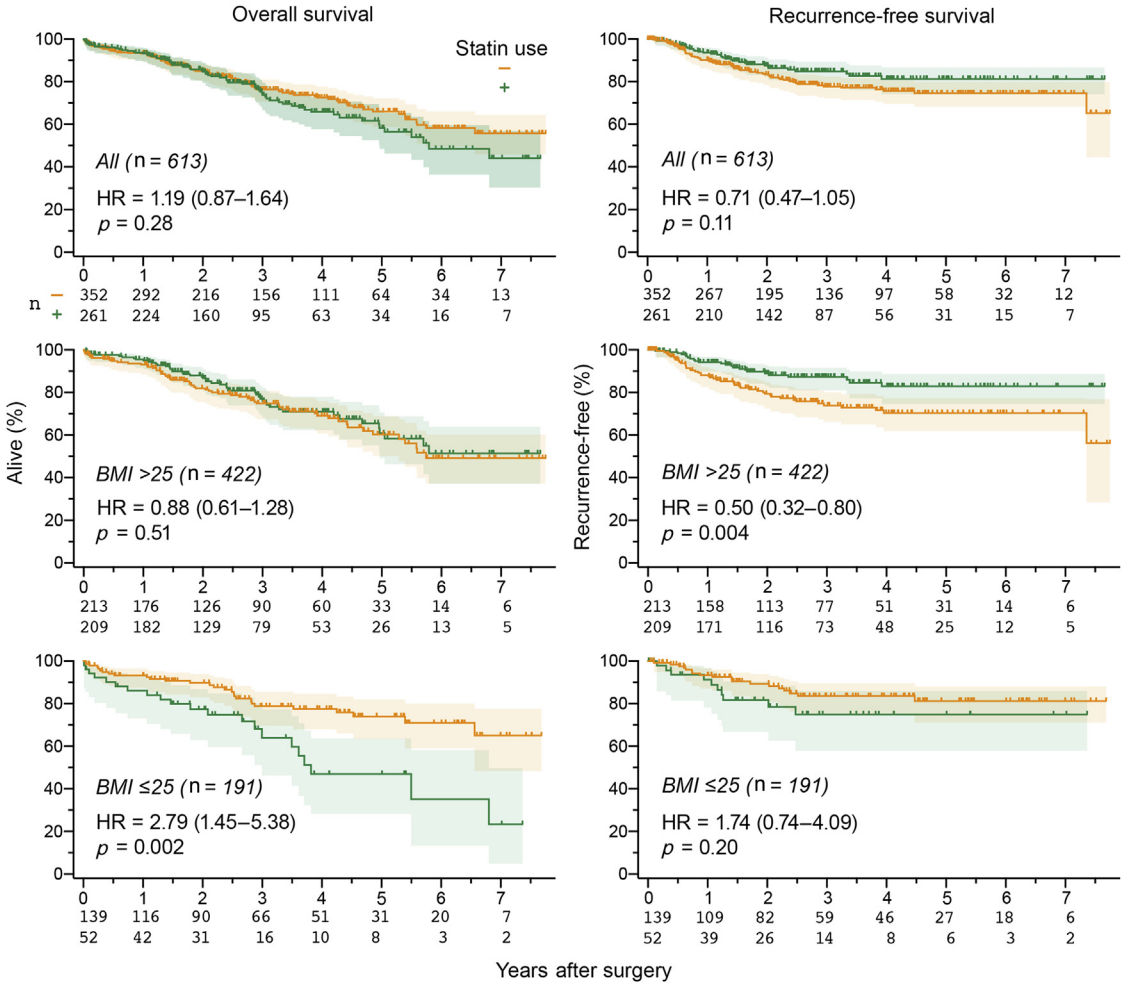

Figure 2. Association of presurgery statin use with survival after lobectomy for early stage NSCLC. Kaplan-Meier curves for overall and recurrence-free survival among subjects using or not using statin before surgery are found for all 613 patients with pathologic stage 1 or 2 or their subgroups of 422 obese/overweight subjects with BMI greater than 25 and 191 subjects with BMI less than or equal to 25. Shaded regions indicate 95% confidence intervals. Subjects at risk at different time points and log-rank test *p* value and HR associated with statin use and its 95% confidence interval are noted. BMI, body mass index; HR, hazard ratio.

Specifically, the risk of recurrence after lobectomy of early stage NSCLC was significantly reduced by statin use in only overweight and obese patients. It is important to note that the proportion of these patients in the study is not trivial (69%). Our finding therefore has implications for not only the design of clinical trials of statins for lung cancer but also the large proportion of patients with lung cancer who can potentially benefit from statin use. It also highlights the importance of considering medications in examinations of the association of obesity with development risks and clinical outcomes of lung cancer. In our cohort, subjects with BMI greater than 25 were four times more likely to be statin users than subjects with BMI less than or equal to 25. The obesity (BMI)-dependent effect of statins that is observed in this study is in line with multiple clinical and preclinical studies revealing obese and nonobese individuals can have different responses to statins. For instance, BMI has been found to affect statin-mediated changes in plasma low-density lipoprotein and C-reactive protein levels and atherosclerotic plaque dimensions,[33] and statin intake affects the gut microbiome of individuals with BMI greater than 30 uniquely compared with individuals with BMI less than 30.[34] Statin users who are obese have reduced mortality during the first year after acute myocardial infarction compared with nonobese statin users.[35]

Our examination of tumor transcriptomes suggests an immunologic underpinning for the obesity-specific benefit of statin use in NSCLC. Specifically, tumors of statin-using high-BMI patients had overexpression of tumoricidal genes such as those encoding for granulysin and interferon-γ compared with statin nonusers. This association of tumor immune gene expression with

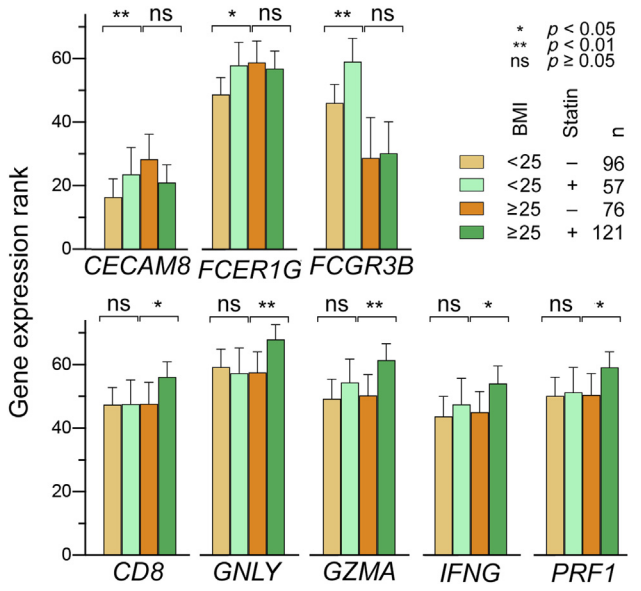

Figure 3. Association of statin use with lung cancer tumor gene expression in patient groups with low and high BMI. Targeted RNA sequencing was used to profile expression of 397 genes in tumors of 350 non-metformin-using patients with lung cancer (both small and nonsmall cell, and all clinical stages). Revealed are mean expression level and its 95% confidence interval for eight selected genes for which differential expression between statin users and nonusers was observed (Wilcoxon ranked sum test $p < 0.05$) in subgroups of patients with BMI less than 25 and greater than or equal to 25. BMI, body mass index; ns, not significant.

statin use was not found in low-BMI patients. In line with previous studies suggesting statins can enhance suppressive myeloid cell populations,[36] we observed a significant, drug-associated up-regulation of genes associated with myeloid-derived suppressor cells but only in low-BMI patients. This association was absent in high-BMI patients. Although the induction of suppressive myeloid cells may have desirable effects, such as enforcement of immune tolerance in an autoimmune or inflammatory disease context, in the cancer setting, such action can be expected to result in a major obstacle facing effective antitumor immunity. These observations suggest that statins can facilitate a unique and desirable effect on the antitumor immune defenses in the obese setting. It is possible that the immunostimulatory effects of statins in the high-BMI context, including the enhanced production of tumoricidal interferon-γ, granulysin, granzyme A, and perforin 1, account for the improved RFS found in our study.

Our study has multiple important limitations, including its retrospective nature, exclusion of late-stage disease in the survival analyses, the lack of an independent validation cohort, and the definition of statin exposure. The focus on patients with early stage NSCLC undergoing a lobectomy was intended to decrease treatment-related variability in recurrence. It is very much possible that statin exposure merely indicates an underlying disease state for which the drug was originally prescribed (e.g., hypercholesterolemia), and the apparent anticancer benefit in some individuals or subpopulations may be tied to the biology of these underlying conditions that led to statin therapy or their correction. Randomized controlled studies will be required to address this. In our study, we defined statin exposure as that occurring at the time of lobectomy. It is entirely possible that patients in the statin-nonexposed group had started using statins within a few months after surgery. The converse may be true as well, with patients in the statin-exposed group receiving statin therapy at the time of surgery but stopping it within a few months. Indeed, in routine practice, a significant proportion of patients who are prescribed statins are not maintained on the therapy beyond a certain period,[37,38] leading to a potentially confounding issue.

We also did not evaluate patient adherence to statin therapy, nor did we evaluate the effect of duration of therapy. Our study also did not evaluate the effect of statins on survival on the basis of their drug class, or on genomic characteristics of patients' tumors. This was because of the relatively small number of outcome events in the study cohort. Statins are not a single drug, and statins of lipophilic class, such as atorvastatin, can have different biological effects than hydrophilic statins, such as pravastatin.[39] Indeed, a recent meta-analysis revealed that the association of statin use with reduced risk of ovarian cancer was greater for lipophilic than hydrophilic statins.[40] The effect of statins on tumors may also be distinct between different types of lung cancer tumors, as suggested by a recent study that observed an effect of *TP53* mutation status on the association of statin use with postresection prognosis of lung adenocarcinoma.[41] An additional study limitation is that it did not consider comorbidities either individually or in aggregate (e.g., Elixhauser index) because pertinent data were unavailable for most patients. Nevertheless, although comorbidities such as diabetes and hypertension will influence OS and are likely to differ in prevalence among statin users and nonusers, they are not expected to have any significant preventive effect on cancer recurrence as was observed for statin use by the overweight/obese patients of our study. The use of two significantly different lung cancer cohorts for separately evaluating association of statin use and obesity with survival and tumor transcriptome is another limitation of our study. Despite these demerits, our finding of reduced NSCLC recurrence among high-BMI statin users is statistically robust with a biological premise provided by our tumor transcriptome analysis, and it provides a rationale for further investigations.

To conclude, in our analysis of a single-center retrospective data set, we find that statin use is associated with improved RFS of overweight/obese patients undergoing lobectomy for early stage NSCLC. We observe that statin use is also associated with markers of heightened antitumor activity in tumors of this patient population. These novel findings suggest that BMI modulates the beneficial effect of statins in NSCLC. If validated, high BMI may be a simple biomarker for selecting patients with NSCLC for anticancer clinical trials of statins.

CRediT Authorship Contribution Statement

Santosh K. Patnaik: Formal analysis, Methodology, Visualization, Writing—original draft.

Cara Petrucci: Formal analysis.

Joseph Barbi: Formal analysis, Writing—review and editing.

Robert J. Seager: Formal analysis, Visualization, Writing—original draft.

Sarabjot Pabla: Data curation, Methodology, Writing—original draft.

Sai Yendamuri: Conceptualization, Data curation, Resources, Supervision, Writing—original draft.

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *JTO Clinical and Research Reports* at www.jtocrr.org and at https://doi.org/10.1016/j.jtocrr.2021.100254.

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