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Deep Phenotyping of Patient Cohorts with Thoracic Malignancies

Dissertation
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Date of oral defense:	12.01.2021

AFFIDAVIT

I hereby declare, that the submitted thesis entitled

"**Deep phenotyping of patient cohorts with thoracic malignancies**"

is my own work. I have only used the sources indicated and have not made unauthorized use of services of a third party. Where the work of others have been quoted or reproduced, the source is always given.

I further declare that the submitted thesis or parts thereof have not been presented as part of an examination degree to any other university.

Heidelberg, 12.01.2021	Laura Klotz
Place, date	Signature of doctoral candidate

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LIST OF ABBREVIATIONS

ALK	Anaplastic Lymphoma Kinase
COPD	Chronic Obstructive Pulmonary Disease
EGFR	Epidermal Growth Factor Receptor
EML4	Echinoderm Microtubule-associated protein-Like 4
GLAD	Gauting locoregional Lung ADenocarcinoma cohort
IASLC	International Association for the Study of Lung Cancer
KRAS	Kirsten rRAt Sarcoma oncogene
LACE	Lung Adjuvant Cisplatin Evaluation study
LADERS	Lung Adenocarcinoma DEath Risk Score
LADC	Lung ADenoCarcinoma
N	lymph node (TNM staging system)
NSCLC	Non-Small Cell Lung Cancer
SCLC	Small Cell Lung Cancer
TNM	Tumor Nodus Metastasis (Staging System)

PUBLICATIONS INCLUDED IN THE THESIS

Peer-reviewed publications

Comprehensive clinical profiling of the Gauting locoregional lung adenocarcinoma donors.

Klotz LV, Courty Y, Lindner M, Petit-Courty A, Stowasser A, Koch I, Eichhorn ME, Lilis I, Morresi-Hauf A, Arendt KAM, Pepe M, Giopanou I, Ntaliarda G, Behrend SJ, Oplopoiou M, Gissot V, Guyetant S, Marchand-Adam S, Behr J, Kaiser JC, Hatz RA, Lamort AS, Stathopoulos GT.

Cancer Med. 2019 Apr;8(4):1486-1499. doi: 10.1002/cam4.2031.

Prognostic relevance of regional lymph-node distribution in patients with N1-positive non-small cell lung cancer: A retrospective single-center analysis.

Eichhorn F, **Klotz LV**, Muley T, Kobinger S, Winter H, Eichhorn ME.

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INTRODUCTORY SUMMARY

1. Lung cancer

Lung cancer is the second leading cause of cancer death in women and the most common cause of cancer death in men [1]. Smoking still remains the predominant cause of lung cancer development, but in addition environmental factors like air pollution, radon, and asbestos as well as genetic susceptibility are of increasing relevance [2]. Lung cancer can be histologically classified as Non-small Cell Lung Cancer (NSCLC) and Small Cell Lung Cancer (SCLC). In Germany, about 80% of all lung cancer cases are NSCLC [3].

NSCLC is a heterogeneous tumor entity with its two main subtypes of squamous cell carcinoma and adenocarcinoma. In recent years, lung adenocarcinoma (LADC) has become the most common histologic subtype of lung cancer [4, 5]. LADC is responsible for approximately 650.000 deaths per year worldwide and represents the most deadly form of human cancer [6]. Regardless of the smoking status, the incidence of LADC is still increasing worldwide [1, 6, 7]. In addition, LADC represents the most frequent histological subtype of lung cancer in women, younger people, and never smokers [7]. Therefore, there is an important need to understand the reasons for the development of LADC by phenotyping and genetically characterizing the disease to be able to develop personalized and effective treatment strategies.

As described, known causes of LADC are smoking and radon exposition, but since this deadly disease is getting more frequent in never-smokers and young patients, other relevant factors like genetic mutations or environmental influences have to be causative. In recent years, so called driver mutations have been identified in NSCLC, primarily in never smokers or ex-smokers with LADC. The two most relevant driver mutations in the clinical routine are a) activating mutation of the epidermal growth factor receptor gene (EGFR) and b) fusion of the echinoderm microtubule-associated protein-like 4 (EML4) gene with the anaplastic lymphoma kinase gene (ALK) [8].

2. Challenges in the treatment of lung cancer

Although multiple approaches concerning cancer prevention and early detection of cancer development have been established, only about 15% of the patients diagnosed with locoregional LADC have the option to undergo surgical tumor resection with curative intent if distant or contralateral metastases can be ruled out [9, 10]. This is due to a long asymptomatic period of lung cancer growth until late in the disease development. Moreover, pulmonary symptoms of many smokers are often non-specific, leading to a clinically relevant delay of lung cancer diagnosis and start of adequate cancer treatment [11].

Unfortunately, there is a relevant proportion of about 10 to 15% of the patients suffering from locoregional tumor recurrence after complete surgical resection of an initially early-stage NSCLC [12]. Furthermore many patients suffer from distant metastases during follow-up, resulting in a limited prognosis [13]. Additional adjuvant chemotherapy can reduce tumor recurrence following surgery for localized lung cancer with a tumor size larger than four centimeters or lymph node metastases [14]. The Lung Adjuvant Cisplatin Evaluation (LACE) study investigated the effect of platinum-based adjuvant chemotherapy following resection of early stage NSCLC and identified an increased 5-year survival of 5.4% for patients receiving adjuvant chemotherapy [15]. Nevertheless, due to the individual therapeutic response of each patient to chemotherapy, it remains impossible to predict which patients will benefit from adjuvant chemotherapy [15].

3. Aim of the research project

Current molecular findings indicate that LADC might be a different disease entity within the heterogeneous group of NSCLC [16]. To date, the cell of origin and different relevant cellular processes during carcinogenesis concerning LADC are still under debate [17]. Airway cells play a central role for lung regeneration and carcinogenesis because epithelial developmental steps seem to be linked with important oncogenic signaling pathways which are involved in the development of LADC [17-19].

Although novel mutations are constantly discovered by extensive analyses of tumor tissue, their phenotypic impact and especially their potential clinical significance are largely ambiguous [20, 21]. To help address this issue, global genomic information of carefully clinically phenotyped patient cohorts with LADC would be invaluable. A precise phenotyping might help to improve the knowledge of the complex pathobiology of LADC and to optimize personalized treatment strategies [22]. Possible associations of clinical variables of LADC patients with molecular biomarkers have not been extensively analyzed in large patient cohorts.

A detailed correlation of the phenotype and genotype of LADC may contribute to improve personalized targeted therapy for patients with defined oncogenic driver mutations [23]. Based on this, we aim to identify associations of patient characteristics and molecular biomarkers and to describe novel tumor genome-phenome links which might be of therapeutic relevance.

3.1 Important aspects of the research project

In the publication “Comprehensive clinical profiling of the Gauting locoregional lung adenocarcinoma donors”, we analyzed substantial amounts of clinical information of 366 patients undergoing surgery for locoregional LADC with curative intent at the Lung Clinic Gauting. Clinical parameters were reported with focus on patient characteristics, perioperative data and follow-up information concerning recurrence-free and overall survival [24]. Based on this cohort, clinical parameters could be correlated to individual pathological and genetic alterations of the tumor tissue and the tumor environment. For validation of the surgical cohort, clinical findings were evaluated in another cohort from France and in comparison to previous studies.

In our analysis, we were able to confirm the high frequency of LADC in women, never smokers, and ex-smokers [25]. The data underlines that smoking is closely correlated with the development of LADC and COPD, suggesting that active smoking promotes the ongoing tumor growth in lung tissue [26, 27]. Initial results from our surgical cohort of patients with locoregional LADC support the value of the revised 7th TNM staging system and of the LADC histologic classification system [28-30].
We could show novel clinical associations such as the predominance of these tumors to arise in the right upper lobe for smokers. Solid tumor subtype is more frequent in smokers while never smokers frequently show acinar tumor histology [24]. Smoking status is negatively associated with lymph node involvement, pleural spread, and bone metastases during follow-up [24]. Interestingly, overall survival was significantly reduced in patients younger than 45 years when compared to patients with an age between 45 and 65 years. Possibly, younger patients develop a more aggressive tumor type which might be due to germline tumor suppressor loss. This hypothesis will be tested and analyzed in the matching LADC tumor tissue in upcoming projects [31].

For the first time, our work substantiated that time from diagnosis to surgery represents a crucial interval regarding overall survival, enhancing the aggressive growth pattern of LADC and the important value of surgery in a timely manner for the treatment of locoregional disease. In addition, we identified differences in the temporal development of organ-specific metastases, probably comparable to biphasic metastatic patterns of other solid tumors like colorectal or gynecological cancer [32]. According to this, we could demonstrate that pleural metastases and pleural effusion develop earlier during follow-up than ipsilateral or contralateral pulmonary metastases [24].

Using clinical characteristics of the individual patient and follow-up data regarding disease-free and overall survival, different clinical factors such as the adverse effects of age for patients younger than 45 or older than 65 years, FEV_1 below 80% of the age-appropriate set point, and delayed resection on survival defined as surgery later than 60 days after diagnosis, could be identified to substantially affect prognosis. In accordance with the described clinical work and our research interest, we utilized the detailed and valuable clinical information from our cohort for the establishment of a new lung adenocarcinoma death risk score (LADERS) in order to predict overall survival after surgery for locoregional disease [24]. This clinical score is clearly structured without the need for additional clinical tests, and represents an accurate clinical classification system to assess overall survival for patients with locoregional LADC.

The independent predictors of survival were identified by proportional hazards Cox regression analysis. Based on this, the locoregional lung adenocarcinoma death risk score was calculated as follows.

Variable	**Hazard ratio** (95% confidence interval)	**Probability**	**Hazard points**
Age < 45 or > 65 years	4.12(2.35-7.23)	0.0000008	3
FVC < 80% predicted	2.13(1.25-3.63)	0.0054374	1
DL_{CO}/V_A < 70% predicted	2.62(1.60-4.29)	0.0001292	2
N2	3.56(2.20-5.76)	0.0000002	2.5
N3	8.65(1.10-68.21)	0.0406576	7.5
Time to surgery > 60 days	4.04(2.07-7.88)	0.0000408	3
Solid histologic subtype	2.09(1.27-3.43)	0.0035422	1
		LADERS	0-20

The LADC risk score was able to predict overall survival more precisely compared to the 7th TNM staging system. The 7th TNM staging system was still superior in predicting recurrence-free survival. Consequently, a combination of detailed clinical information together with histopathological analysis represents a promising approach to uncover novel genome-phenome links in LADC and might achieve valuable insights into the mechanisms of carcinogenesis in the respiratory tract.

4. Aim of the second research project

The work within the manuscript “Prognostic relevance of regional lymph-node distribution in patients with N1-positive non-small cell lung cancer: A retrospective single-center analysis” was conducted with the aim to specify the classification system of lung cancer staging

concerning lymph node involvement [33]. The current staging system has been developed and improved by the International Association for the Study of Lung Cancer (IASLC) based on a large patient cohort mainly from Asian countries [34]. Since it cannot be ruled out that the prognostic factors for survival are different in Asian and European patients, the prognostic factors for survival should be validated in a large cohort of patients from Europe with locoregional lung cancer and involvement of hilar lymph nodes (N1 positive) [33]. According to this, we analyzed 317 patients undergoing surgery for lung cancer with curative intent at the Thoraxklinik Heidelberg. Clinical associations between the presence of lymph node metastases in the peripheral or hilar zone (N1 position) of the resected lung lobe and overall survival were retrospectively analyzed.

4.1 Important aspects of the research project

In the publication “Prognostic relevance of regional lymph-node distribution in patients with N1-positive non-small cell lung cancer”, we retrospectively analyzed clinical data of patients with N1-positive NSCLC undergoing curative-intent thoracic surgery. In case of advanced locoregional disease with tumor metastases in hilar (lymph node positions 10-11) and/ or peripheral (lymph node positions 12-14) lymph nodes of the N1 compartment, patients with LADC have a poor prognosis compared to patients without lymph node metastases. According to the different N-descriptors of the current TNM staging system, overall survival of the individual patient with locoregional disease is dependent on the presence of hilar or mediastinal lymph node metastases [34]. However, significant differences concerning disease-free and overall survival have been identified for lung cancer patients within the same N-category [35]. Analysis of the IASLC cohort of N1-positive patients revealed a decrease of overall survival in correlation with an increasing number of metastatic lymph nodes. Consequently, the IASLC committee gave the recommendation to subdivide N1-positive lung cancer patients into N1a (single lymph node position involved) and N1b (more than one position involved) in the upcoming TNM staging classification [34]. We were able to confirm the differences concerning overall survival for patients with pathologically confirmed N1a and N1b lymph node involvement following curative-intent surgery. Due to the fact that there is only limited European patient data available in the IASLC database with only 544 N1 patients from two European countries, our patient cohort with 317 patients displays an important and valuable addition to published clinical data [28, 34]. For detailed classification of lymph node involvement and to validate possible correlations to disease-free and overall survival, Rusch and colleagues proposed the classification of lymph nodes into “zones,” which has been adopted by the IASLC lymph node map [36]. According to this proposal, we

could verify that lymph node metastases in more than one zone were associated with a poor overall survival compared to lymph node involvement in one zone [33].
Currently, adjuvant treatment decisions are based on the postoperative, pathological lymph node status with no regard to the individual nodal tumor burden. Based on the observed differences in survival depending on involved lymph node subgroups, future therapy trials should aim at analyzing a possible impact of adjuvant and neoadjuvant treatment regimens based on more detailed patient stratification depending on single lymph node stations. New therapeutic strategies like targeted therapy and immunotherapy might increase disease control for patients with locoregional disease and hilar lymph node involvement [37, 38]. Taken together, the involvement of the different lymph node positions could be of prognostic relevance for the evaluation of the tumor immune response and development of effective new therapeutic strategies.

5. Contribution of the PhD candidate

Concerning the publication “Comprehensive clinical profiling of the Gauting locoregional lung adenocarcinoma donors”, the PhD candidate designed the project together with her supervisor Dr. Georgios Stathopoulos. The PhD candidate acquired the clinical data from medical charts, follow-up visits, and phone calls. Statistical analyses were conducted by the PhD candidate together with her supervisor. Moreover, the PhD candidate wrote the first version of the manuscript.

Regarding the publication “Prognostic relevance of regional lymph-node distribution in patients with N1-positive non-small cell lung cancer”, the PhD candidate collected and analyzed clinical data and performed statistical analysis.

PUBLICATION I

Comprehensive clinical profiling of the Gauting locoregional lung adenocarcinoma donors

Klotz LV, Courty Y, Lindner M, Petit-Courty A, Stowasser A, Koch I, Eichhorn ME, Lilis I, Morresi-Hauf A, Arendt KAM, Pepe M, Giopanou I, Ntaliarda G, Behrend SJ, Oplopoiou M, Gissot V, Guyetant S, Marchand-Adam S, Behr J, Kaiser JC, Hatz RA, Lamort AS, Stathopoulos GT.

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ORIGINAL RESEARCH

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Comprehensive clinical profiling of the Gauting locoregional lung adenocarcinoma donors

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Abstract

A comprehensive characterization of lung adenocarcinoma (LADC) clinical features is currently missing. We prospectively evaluated Caucasian patients with early-stage LADC. Patients with LADC diagnosed between 2011 and 2015 were prospectively assessed for lung resection with curative intent. Fifty clinical, pathologic, radiologic, and molecular variables were recorded. Patients were followed till death/study

Laura V. Klotz, Anne-Sophie Lamort and Georgios T. Stathopoulos are equally contributing authors.

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conclusion. The main findings were compared to a separate cohort from France. Of 1943 patients evaluated, 366 were enrolled (18.8%; 181 female; 75 never-smokers; 28% of registered Bavarian cases over the study period). Smoking and obstruction were significantly more prevalent in Gauting Lung Adenocarcinoma Donors (GLAD) compared with adult Bavarians ($P < 0.0001$). Ever-smoker tumors were preferentially localized to the upper lobes. We observed 120 relapses and 74 deaths over 704 cumulative follow-up years. Median overall and disease-free survival were >7.5 and 3.6 years, respectively. Patients aged <45 or >65 years, resected >60 days postdiagnosis, with abnormal FVC/$DL_{CO}V_A$, N2/N3 stage, or solid histology had significantly decreased survival estimates. These were fit into a weighted locoregional LADC death risk score that outperformed pTNM7 in predicting survival in the GLAD and in our second cohort. We define the clinical gestalt of locoregional LADC and provide a new clinical tool to predict survival, findings that may aid future management and research design.

KEYWORDS
LADC, lung adenocarcinoma, obstruction, smoking, survival

1 | INTRODUCTION

Lung adenocarcinoma (LADC) is the most frequent histologic type of lung cancer.[1,2] It constitutes the most deadly human cancer, causing 650 000 deaths per year worldwide,[3,4] while its incidence is increasing in active smokers, ex-smokers, and never-smokers.[5] Simultaneously, LADC is the most frequent lung cancer in never-smokers, women, and young patients, rendering understanding and treating the disease imperative.[5,6] LADC is mainly caused by smoking, radiation, and other exposures.[5,7] Although multiple approaches to prevention/early detection have been evaluated, only 15% of patients diagnosed with LADC are amenable to surgery, the only definitive cure.[8] These patients are of tremendous importance, since they donate tissues for research that has fostered our understanding of the pathobiology of locoregional LADC and has enabled targeted therapies for patients with defined oncogenic driver mutations.[9,10]

Recent molecular evidence indicates LADC to be a distinct disease entity.[11] However, the clinical gestalt of the disease has not been comprehensively characterized separately from other forms of lung cancer. Here we report the first results from the Gauting locoregional lung adenocarcinoma donors (GLAD) study, a prospective biobank of LADC tissues and clinical phenotypes. The wealth of clinical information provided includes multiple variables and prolonged follow-up data, enabling the discovery of new associations reported here, as well as the future establishment of genotype-phenotype links.

2 | MATERIAL AND METHODS

2.1 | Studies approval

GLAD was conducted in accord with the Helsinki Declaration, reported in accord to STROBE (https://www.strobe-statement.org/index.php?xml:id=strobe-home), approved by the LMU Ethics Committee (623-15), registered with the German Clinical Trials Register (http://www.drks.de/drks_web/navigate.do?navigationId=trial.HTML&TRIAL_ID=DRKS00012649), and written informed consent was obtained from all patients (https://www.asklepios.com/gauting/experten/experten/biobank/). The Tours study was conducted according to the Helsinki Declaration, was approved by the Ethics Committee of Région Centre (2015/051), and was registered with the French Ministry of Health (DC-2008-308). All patients gave written informed consent.

2.2 | GLAD study

All patients with histologic LADC diagnosis at Asklepios Medical Center between February 2011 and September 2015 were prospectively evaluated for lung resection with curative intent. LADC was staged according to the current Seventh Edition of the International Association for the Study of Lung Cancer (IASLC) tumor–node–metastasis staging system (TNM7).[2] Preoperative lung function was assessed according to current guidelines.[12] The Absolute

and percentage predicted values for forced vital capacity (FVC), forced expiratory volume in 1 sec (FEV_1), FEV_1/FVC ratio, lung diffusion capacity for carbon monoxide (DL_{CO}), and DL_{CO} corrected for alveolar ventilation (DL_{CO}/V_A) were recorded. Patients eligible and fit for surgery were prospectively enrolled. Baseline data obtained at entry were: blinded patient identifier (ID), age and sex, body mass and length, date and mode of clinical and tissue diagnosis, clinical TNM7 (cTNM7) stage including site and extent of metastatic disease, smoking start, stop, and intensity, and lung function results. Chronic obstructive pulmonary disease (COPD) was defined as smoking >30 pack-years with compatible symptoms and FEV_1/FVC <70% and was graded by the global initiative for chronic obstructive lung disease (GOLD) 2001 classification.[13] All patients were re-evaluated at 30 days postsurgery, the benchmark of referral to oncology/radiotherapy (all stage III/IV patients received adjuvant therapy) or dismissal to out-patient follow-up according to current guidelines.[14] Data prospectively recorded included: date of surgery, time from diagnosis to treatment calculated from imaging/tissue diagnosis (whichever occurred first) to resection date, blinded tissue ID, lobar tumor location, relapse/metastasis date and site, histologic subtype, pathologic TNM7 (pTNM7) stage, and oncogene testing results. Follow-up data were retrospectively acquired from visits, medical charts, telephone consultations with treating physicians, and/or death certificate searches and included: adjuvant therapy, relapse/metastasis date, site, and extent, and death or last contact. Primary endpoint was overall survival (OS), calculated from surgery to death (event) or last contact (censored); secondary endpoint was disease-free survival (DFS), calculated from surgery until recurrence (event) or last contact (censored); tertiary endpoints were associations between the variables obtained.

2.3 | Tours comparison cohort

All patients with tissue-diagnosed LADC between January 2006 and December 2011 were prospectively evaluated for curative resection, staged according to TNM7,[2] preoperatively tested for lung function, prospectively enrolled if eligible, and fit for surgery. Data obtained and endpoints were identical to GLAD, except from histologic subtype, extent of metastatic disease, and oncogene test results.

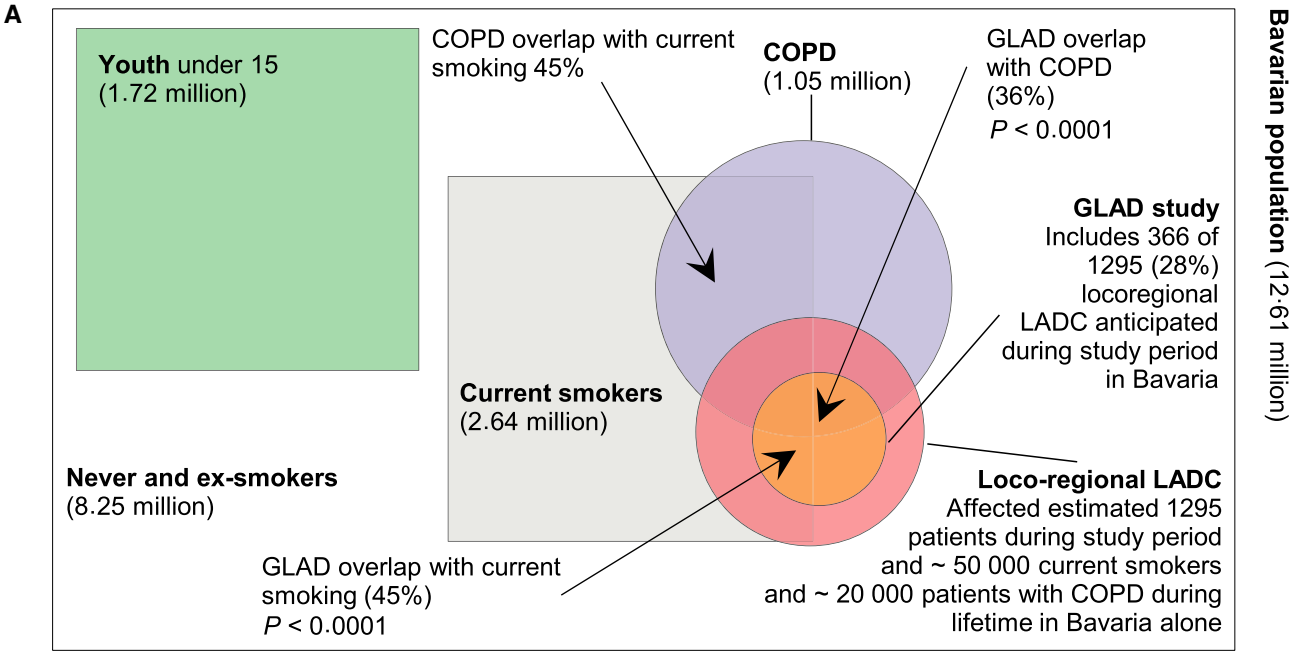
2.4 | Histology and genotyping

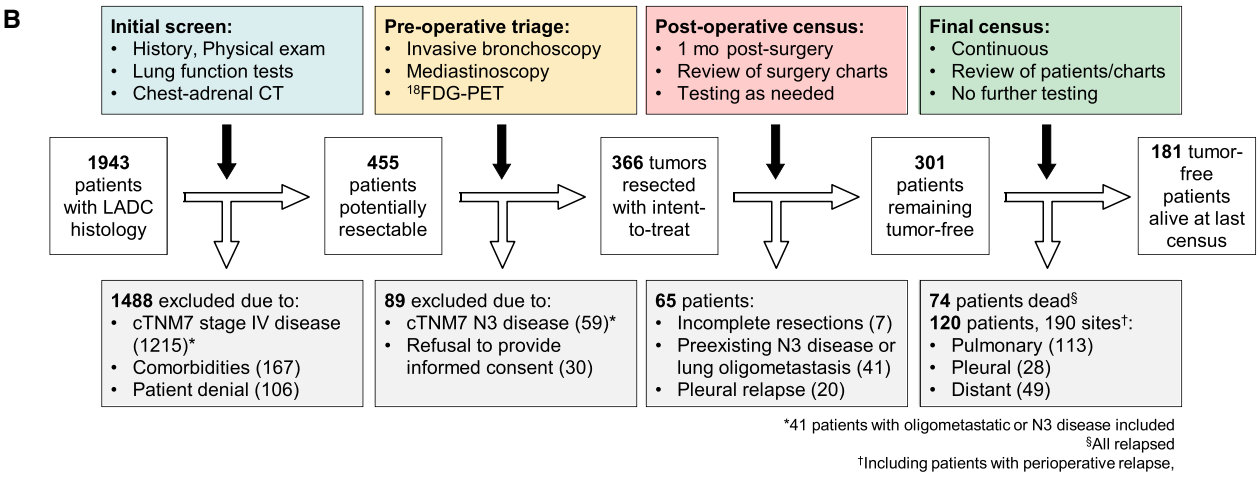
LADC subtypes of GLAD were determined by our pathology expert (AMH) according to IASLC guidelines.[1,2]

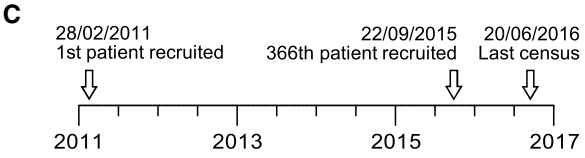
2.5 | Statistics

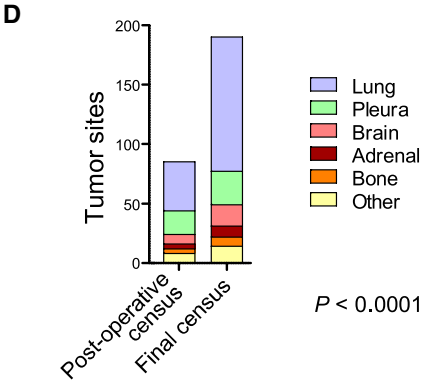
Minimal study size (n^{MIN}) was determined by power analyses (http://www.gpower.hhu.de/en.html) employing Fisher's exact test, proportion inequalities in two independent groups, α error=0.05, 80% power, and 1:1 allocation ratio. $n^{MIN} = 314$ was required to detect the difference between 0% and 5% and $n^{MIN} = 348$ between 30% and 45%. We targeted recruitment to $n = 350$ and achieved $n = 366$ in September 2015. Data distribution was tested using Kolmogorov-Smirnov test and summaries are given as frequencies or point estimates (mean or median) with descriptors of dispersion (standard deviation, SD or interquartile range, IQR or 95% confidence interval, 95%CI), as appropriate and indicated. Survival was analyzed by Kaplan-Meier estimates and Cox proportional hazard models using Waldman backward elimination. Log rank tests were used for comparisons. Associations between variables were examined using Fisher's exact or χ^2 tests, Student's *t*- or Mann-Whitney *U*-tests, one-way analysis of variance (ANOVA) with Bonferroni posttests or Kruskal-Wallis ANOVA with Dunn's posttests, Pearson's or Spearman's correlations, and linear regression, depending on input and target variable nature and distribution, as appropriate and as indicated. Probabilities (P) < 0.05 were considered significant. Least absolute shrinkage and selection operator (LASSO) regression analysis was carried out using the GLMNET package on R*, where the number of regression coefficients shrunk according to a penalization factor λ (https://www.r-project.org/) and their point estimates were determined with cross-validation using 244 samples with complete records. Unsupervised clustering of 362 GLAD patients was done using ConsensusCluster;[15] settings were $K = 2$-6, subsample size = 300, and fraction = 0.8, *K*-means algorithm with average linkages, hierarchical consensus, and Euclidean distance metric, and center principal component analysis normalization with fraction = 0.85 and eigenvalue weight = 0.25. Receiver-operator curves (ROC) were used to identify variables defining patient clusters. Analyses were done on the Statistical Package for the Social Sciences v24.0 (IBM, Armonk, NY) and Prism v5.0 (GraphPad, San Diego, CA).

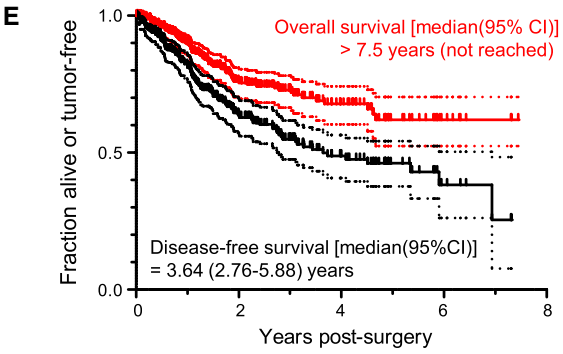
FIGURE 1 The Gauting locoregional lung adenocarcinoma donors (GLAD) study overview and main results at the mid-2016 census. (A) Venn diagram of current smoking and COPD prevalence, and LADC incidence over the GLAD study period in Bavaria. Data were obtained from the present study, from the Bavaria cancer registry, and from references 16-18. (B) Study flowchart. (C) Study timeline. (D) Cumulative relapse events observed by site at the 30-day postresection and long-term follow-up benchmarks. Shown are number of observations (n) and χ^2 test probability (P). (E) Kaplan-Meier plots and estimates of overall and disease-free survival with patient numbers at risk, events observed, and patients censored (graph) and actual (excluding censored observations) percentage of patients surviving at 1, 2, 3, 5, and 7 years postresection (table)











n at risk	366 366	138 108	50 40	3 3	0 0	
n dead/relapsed	0 0	61 140	71 159	74 163	74 164	
n censored	0 0	167 118	245 167	289 200	292 202	

Years post-surgery	0	1	2	3	5	7
% alive	100	90	76	70	62	62
% tumor-free	100	69	54	46	39	22

3 | RESULTS

3.1 | The Gauting locoregional lung adenocarcinoma donors (GLAD)

During the period from February 2011 to September 2015, 1943 patients with LADC were prospectively assessed in the Asklepios Medical Center, Gauting, Germany. Among them, 455 were eligible and fit for curative surgery, and 366 were enrolled (89 patients were excluded due to cTNM7 N3 disease or unwillingness to provide informed consent). They represent ~28% of registered Bavarian locoregional LADC cases during the study period (21 588 lung cancer cases, corresponding to 8635 LADC cases at expected 40%, and to 1295 resectable LADC cases at expected 15%; http://www.krebsregister-bayern.de/index_e.html) summarized in Figure 1A.[16-18] During the same period, another 1577 patients with LADC were not eligible or fit for lung resection, rendering 23% of patients screened resectable with intention to treat, and resulting in 19% recruitment rate into GLAD (Figure 1B and C). Of the 366 patients resected, 41 had oligometastatic disease detected prior to surgery, seven were incompletely resected, and in 20 a malignant pleural disease was identified intraoperatively. Out of the 305 patients that were tumor-free after surgery, 301 remained tumor-free at the 30-day postoperative census (82.2%), and 181 (49.5%) at the mid-2016 census (Figure 1D). At this time, 8453 cumulative follow-up months (median[interquartile range, IQR] 18 [7-33] months/patient) had been delivered, and 120 relapses and 74 deaths were observed. Median(95% confidence interval, CI) overall survival (OS) was not reached (>7.5 years), disease-free survival (DFS) was 3.64 (2.76-5.88) years, and 5-year OS and DFS rates were 62% and 39%, respectively (Figure 1E). GLAD will be re-censored mid-biannually; hence survival data are expected to evolve. A color-coded phenome plot of all information available at the mid-2016 census is shown in Figure 2 and Table S1, while a heat map of all the associations observed (discussed below) is given in Figure 3. The major findings from GLAD classified according to clinical variables are presented below.

3.2 | Age

In GLAD, median(IQR) age was 67 (59-72) years, including 11 (3%) and 195 (53%) patients younger than 45 and older than 65 years, respectively; those had markedly decreased overall survival (OS) and disease-free survival (DFS) compared with 160 (44%) patients aged between 45 and 65 years (Figure 4A and B). Age was positively associated with cumulative smoke exposure and lepidic/papillary histology. On the contrary, it was negatively linked with current smoking, body length, FVC and FEV_1, and time to surgery (Figure 3). In addition, more death and relapse events were observed in patients of extreme age (<45 or >65 years) (Figure S1A). Linear regression-calculated lung function decline rates with age were similar to the Framingham study,[19] and lung function test results were tightly correlated with body metric indices, validating GLAD lung function data (Figure S1B-D). Interestingly, patients with affected resection margins and perioperative pleural relapse were significantly younger (Figure S1A).

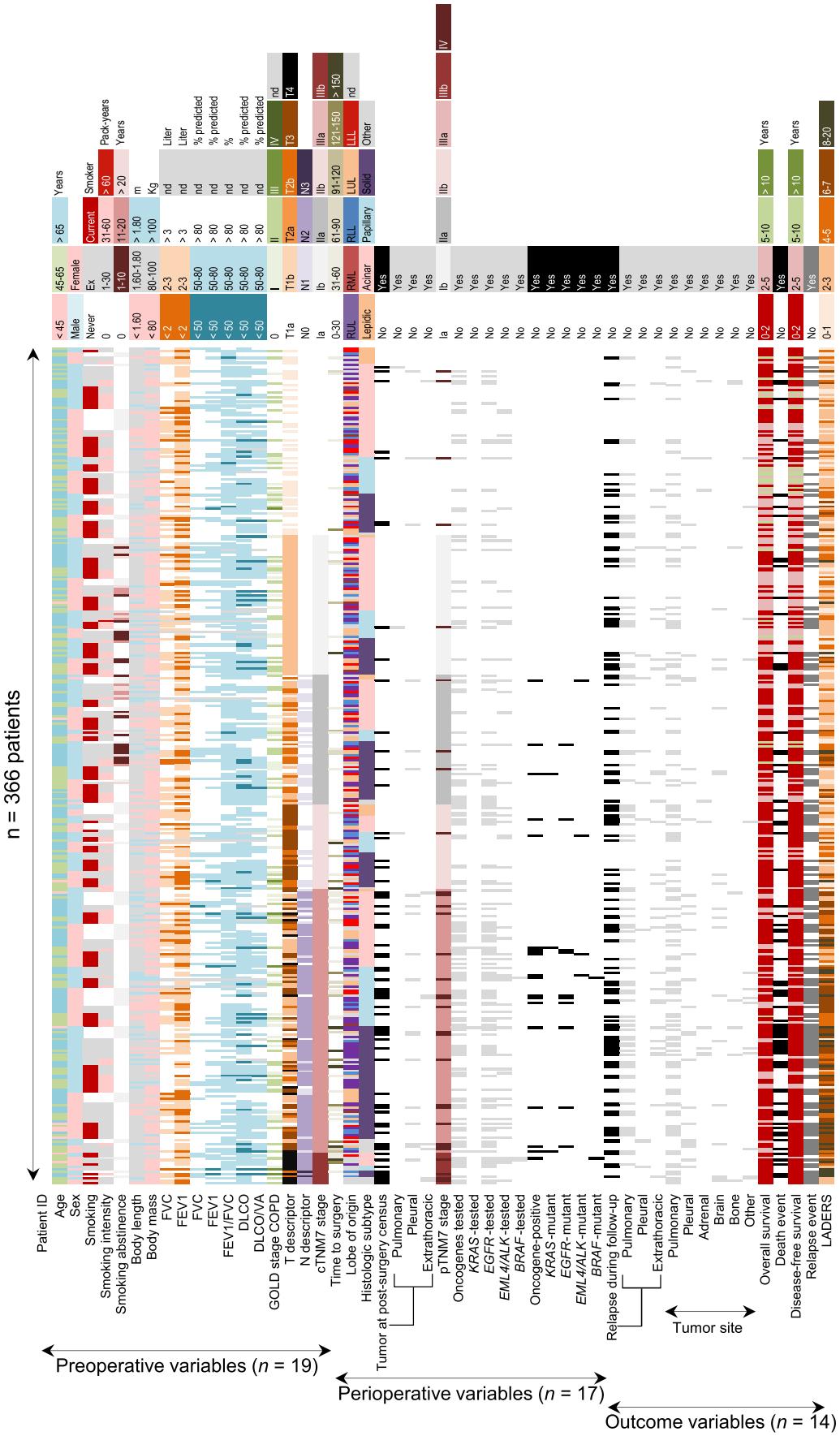
3.3 | Sex

Surprisingly, 181 patients (49.5%) of GLAD were female, reflecting increasing local and worldwide female smoking trends.[6,20] Female sex was positively associated with percent predicted FVC and FEV_1 values and FEV_1/FVC ratio, and negatively linked with smoking rate and intensity, body metric indices, absolute FVC and FEV_1 and percent predicted DL_{CO}/V_A, COPD frequency, solid histologic subtype, and adrenal relapse (Figure S2). However, sex did not significantly impact survival (Figure 4B). These results suggested that locoregional LADC in Caucasian women has distinct features as proposed elsewhere.[6] However, these do not profoundly alter the biologic course of the disease, in accord with published results from Norway.[20]

3.4 | Smoking

The GLAD study included 75 never (20.5%), 130 former (35.5%), and 161 current (44.0%) smokers. Alarmingly, active smokers were younger (Figure 4C). Smoking abstinence of ex-smokers was median(IQR) = 10(5-25) years. Importantly, more than 50% of patients were never/ex-smokers (Figure 1A). GLAD smoking rates were disproportional to a Norwegian cohort of 54 never (7.8%), 255 former (36.8%), and 383 current (55.3%) smokers ($P < 0.0001$

FIGURE 2 GLAD phenome plot. Color-coded pivot table of all data obtained from GLAD sorted sequentially by cTNM7 stage, histologic subtype, sex, and smoking status. Columns represent individual patients and rows variables recorded at study entry, postsurgery census, and longitudinal follow-up. The raw data table is provided as Table S2. *n*, sample size; ID, identifier; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 seconds; DL_{CO}, uncorrected lung diffusion capacity for carbon monoxide; V_A, alveolar ventilation; COPD, chronic obstructive pulmonary disease; GOLD, global initiative for chronic obstructive lung disease; TNM, tumor-node-metastasis staging system; c, clinical; p, pathologic; RUL, right upper lobe; RML, right middle lobe; RLL, right lower lobe; LUL, left upper lobe; LLL left lower lobe; *EGFR*, epidermal growth factor receptor; *KRAS*, V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; *BRAF,* v-Raf murine sarcoma viral oncogene homolog B; *EML4*, echinoderm microtubule associated protein like 4; *ALK*, anaplastic lymphoma kinase; LADERS, locoregional lung adenocarcinoma death risk score; nd, not determined


n = 366 patients
Patient ID
Age
Sex
Smoking
Smoking intensity
Smoking abstinence
Body length
Body mass
FVC
FEV1
FVC
FEV1
FEV1/FVC
DLCO
DLCO/VA
GOLD stage COPD
T descriptor
N descriptor
cTNM7 stage
Time to surgery
Lobe of origin
Histologic subtype
Tumor at post-surgery census
Pulmonary
Pleural
Extrathoracic
pTNM7 stage
Oncogenes tested
KRAS-tested
EGFR-tested
EML4/ALK-tested
BRAF-tested
Oncogene-positive
KRAS-mutant
EGFR-mutant
EML4/ALK-mutant
BRAF-mutant
Relapse during follow-up
Pulmonary
Pleural
Extrathoracic
Pulmonary
Pleural
Adrenal
Brain
Bone
Other
Overall survival
Death event
Disease-free survival
Relapse event
LADERS
Tumor site
Preoperative variables (n = 19)
Perioperative variables (n = 17)
Outcome variables (n = 14)

compared with GLAD, χ^2 test),[20] but proportional to a French cohort from Tours that included 39 never (14.3%), 102 former (37.4%), 124 current (45.4%), and 8 indeterminate (2.9%) smokers ($P = 0.1114$ compared with GLAD, χ^2 test).[21] Hence the Tours cohort was identified as an optimal comparison set (Figure S3, Table S2). Expectedly, current smoking was significantly more frequent ($P = 0.0044$, χ^2 test) in GLAD (44%) compared with current Bavarian rates (24.2%) (Figure 4D).[17] Median(IQR) pack-years smoked were 40 (9-60), and smoking exposure correlated negatively with lung function, especially DL_{CO}/V_A (Figure S4A-C). Moreover, in accord with published results,[22] active smoking was associated with solid, and never smoking with acinar histology (Figure S4D). Interestingly, smoking was negatively associated with N stage, postoperative pleural relapse, as well as bone metastasis (Figure S4D).[9] However, smoking did not affect survival (Figure S4E and F). Collectively the data indicate that smoking is intimately linked with LADC and suggest that active smoking continuously drives the disease in the lungs, likely via tumor-promoting effects of nicotine.[23]

3.5 | Obstruction and COPD

When GLAD were classified according to original GOLD criteria,[13] patients had stage 0 (62.8%), 50 patients stage I (13.7%), 75 patients stage II (20.5%), 6 patients stage III (1.6%), and 5 patients indeterminate (1.4%) COPD status (Figure 2). Smoking was intimately linked with GOLD COPD stage ($P < 0.0001$, Fisher's exact test) and COPD was significantly more prevalent in GLAD compared with current Bavarian rates ($P < 0.0001$, Fisher's exact test; Figure 4D and E).[18] These findings were validated using real-time statistics (https://knoema.com/REG_DEMO_TL2/demographic-statistics?region=1001010-bavaria, http://www.registrecancers59.fr/index.php/incidence) in GLAD and Tours cohorts (Figure 1A, Figure S3A),[24] underpinning the causative role of smoking in both COPD and LADC.[25] Lung function tests were concordant to GOLD COPD definition (Figure S5A). COPD was positively associated with affected resection margins and perioperative pleuropulmonary relapse likely attributable to adverse effects of distorted lung structure on surgical outcome, and correlated negatively with FVC and DL_{CO}/V_A (Figure S5B). However, COPD did not impact survival (Figure S5C and D). Of all lung function variables, only abnormal percentage predicted FVC and DL_{CO}/V_A negatively impacted survival (Figure S5E and F). Collectively, the data indicate that COPD and LADC show significant overlap, suggesting a common pathogenesis, in line with the literature.[25] Moreover, the percentage predicted FVC and DL_{CO}/V_A, but not other spirometry indices or a diagnosis of COPD, can predict survival.

3.6 | cTNM7 staging

All patients were staged according to cTNM7 to guide management.[2,14] We included history, physical exam, and chest-to-adrenal computed tomography in all. For stage III patients, an invasive bronchoscopy with mediastinal lymph node sampling, mediastinoscopy, and/or ^{18}fluoro-deoxyglucose positron emission tomography were also performed. Analysis of T, N, and cTNM7 stage showed a significant impact on survival (Figure S6) and validated GLAD against the reference IASLC study.[2]

3.7 | Surgery

Time from imaging/tissue diagnosis to surgery was median(IQR) = 6 (0-25) days. Resection within 60 days was achieved in 337 patients (92%), while 29 (8%) had resections performed >60 days after diagnosis. Out of 366 GLAD patients, 58 had preexisting oligometastatic, N3 disease, or pleural dissemination newly identified at surgery, leaving 308 for resection with intent-to-treat. Complete resection was achieved in 301 of these patients (97.7%; $P = 0.8639$, Fisher's exact test). Importantly, time to surgery significantly affected overall and disease-free survival (Figure 4F).

3.8 | Tumor location

The lobe of origin of GLAD tumors was definitively determined during surgery in 296 patients, while tumors involving multiple lobes, central airways, and/or mediastinal structures rendered this impossible in 70 patients. We identified a striking upper lobe predominance in both GLAD and Tours cohorts, which was disproportional to published lobe ventilation or perfusion patterns, and was reminiscent of lobar ventilation/perfusion ratios (Figure 4G-I).[26,27] Strikingly, RUL LADCs predominated in smokers of both cohorts, and patients with RUL LADC displayed higher FVC, FEV_1, and N stage, but similar survival, compared with all other patients (Figure S7).

3.9 | Histology

After the pathologic review of multiple tumor sections and sites (AMH), GLAD were classified into 16 lepidic (4.4%), 141 acinar (38.5%), 70 papillary (19.1%), 126 solid (34.4%), 2 fetal (0.5%), 2 adenosquamous (0.5%), 4 micropapillary (1.1%), and 5 indeterminate (1.4%) histologic subtypes. Papillary histology was more frequent compared with a published reference cohort that comprised 41 lepidic (8.2%), 207 acinar (41.4%), 23 papillary (4.6%), 183 solid (36.6%), 33 micropapillary (6.6%), and 13 indeterminate (2.6%) locoregional LADC ($P < 0.0001$, χ^2 test).[22] Encouragingly, indeterminate tumor rate was low in both studies, indicating the reproducibility of the IASLC classification.[1,2,23] In accord with the above-referenced study,[22]

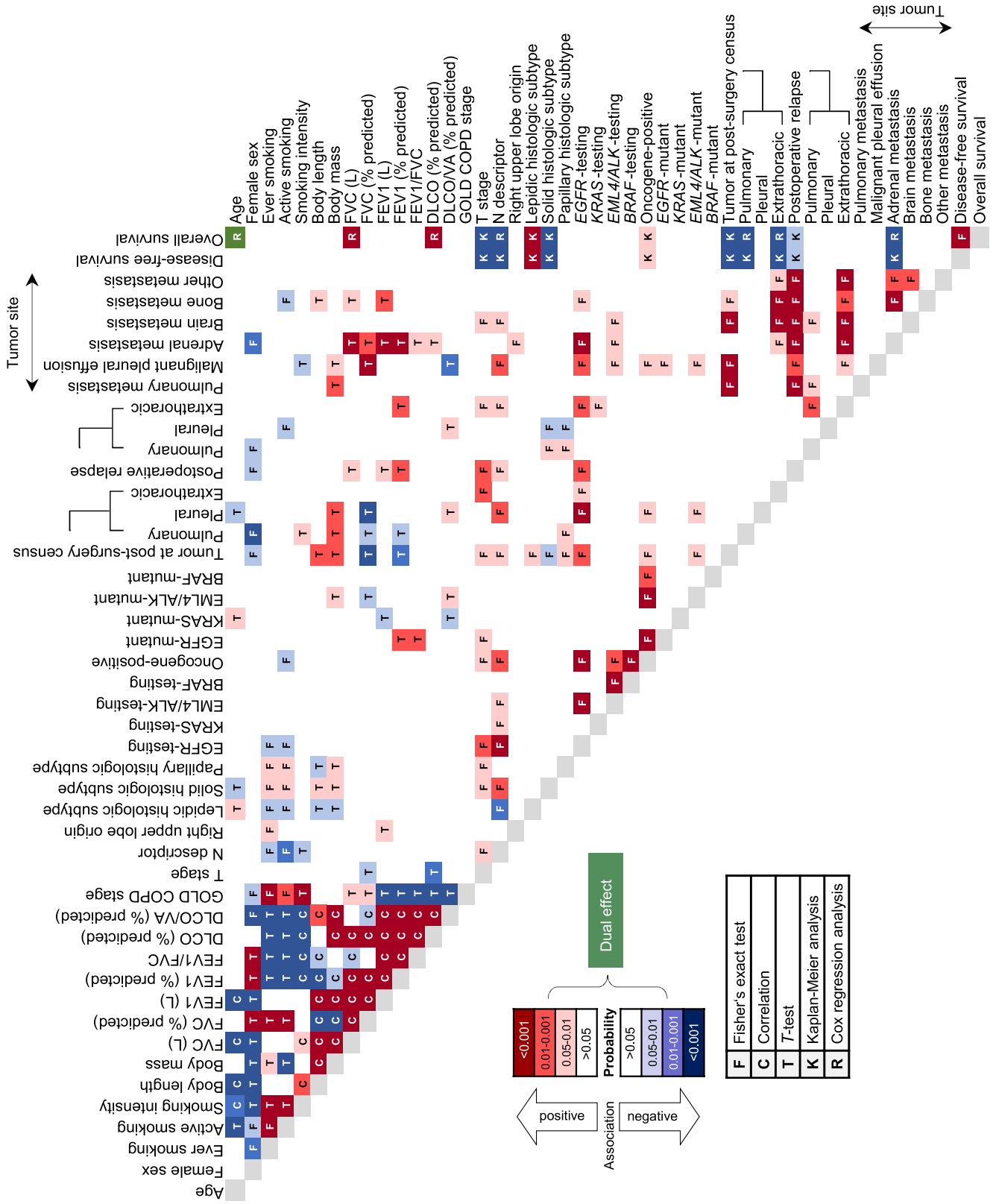


FIGURE 3 GLAD association heatmap. Color-coded pivot table of all associations observed in the GLAD cohort. Colors represent the direction and probability of observed associations and letters the statistical method employed to detect them. *n*, sample size; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 seconds; DL_{CO}, uncorrected lung diffusion capacity for carbon monoxide; V_A, alveolar ventilation; COPD, chronic obstructive pulmonary disease; GOLD, global initiative for chronic obstructive lung disease; TNM, tumor-node-metastasis staging system; c, clinical; p, pathologic; *EGFR*, epidermal growth factor receptor; *KRAS*, V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; *BRAF*, v-Raf murine sarcoma viral oncogene homolog B; *EML4*, echinoderm microtubule associated protein like 4; *ALK*, anaplastic lymphoma kinase

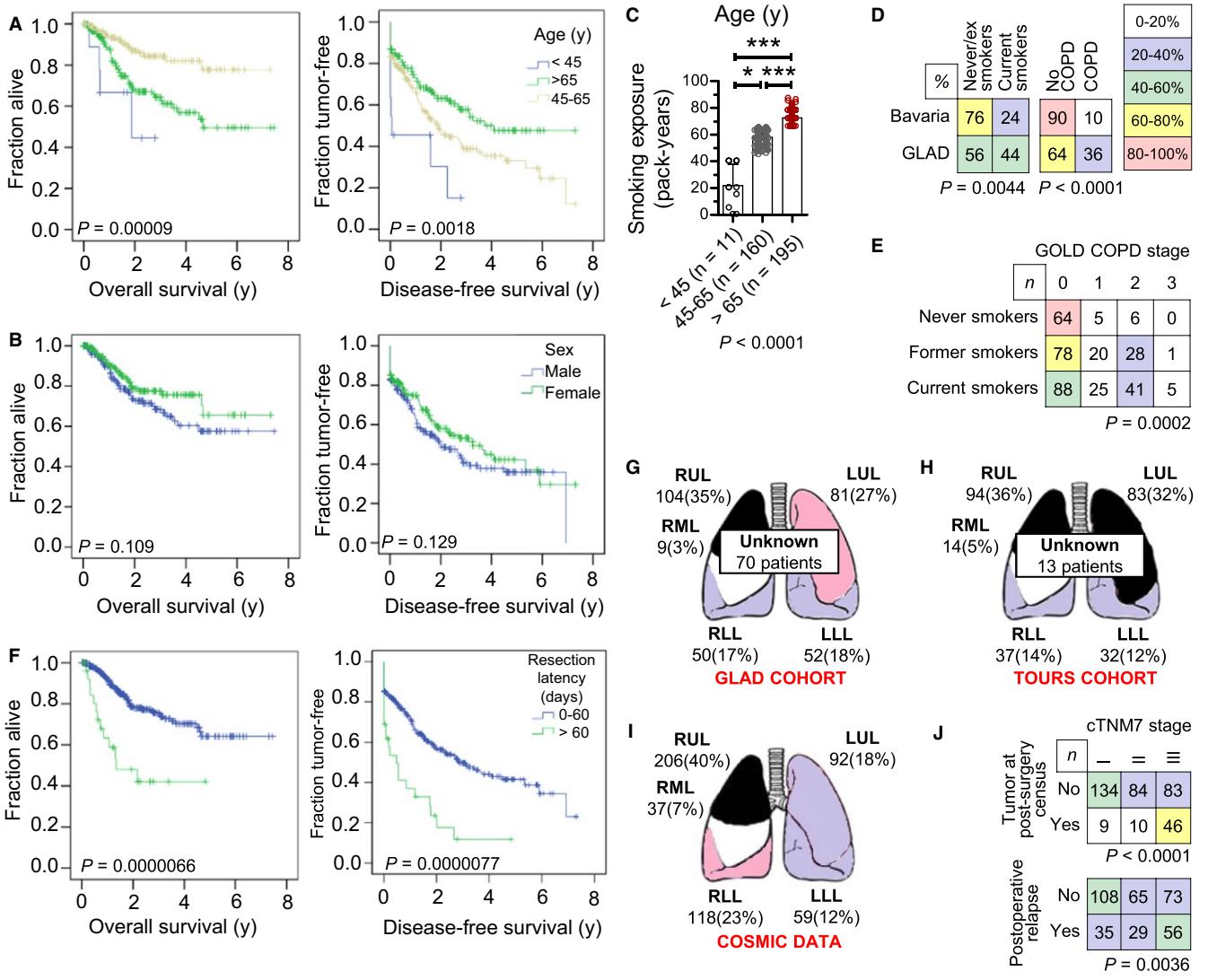


FIGURE 4 Incidence of different clinical parameters on LADC development in GLAD. (A, B, F) Kaplan-Meier disease-free and overall survival plots and overall log-rank test probability values (*P*) of the GLAD. (A) Stratified by age ($n = 11$, 160, and 195, respectively, for age groups <45, 45-65, and >65 years). (B) Stratified by sex ($n = 181$ and 185, respectively, for women and men). (C) Smoking exposure stratified by age. Shown are patient numbers (*n*), raw data points (dots), mean (columns), SD (bars), and Kruskal-Wallis test probability (*P*). * and ***: $P < 0.05$ and $P < 0.001$, respectively, for the indicated comparisons by Dunn's posttests. (D) Crosstabulations of current smoking and COPD prevalence in GLAD and in Bavaria. Data were obtained from the present study and from references 22 and 24. COPD was staged according to the GOLD classification (28). Shown are percentages and Fisher's exact probability (*P*). (E) Crosstabulation of smoking status and GOLD COPD stage in GLAD. Shown are patient numbers (*n*) and χ^2 probability (*P*). (F) Stratified by timely ($n = 337$) or delayed ($n = 29$) resection and by complete ($n = 310$) or incomplete ($n = 56$) resection. (G-H) LADC location by lung lobe determined at surgery (G) in the GLAD derivation cohort, (H) in a smoking-optimal comparison cohort from Tours, France, (I) in COSMIC database (https://cancer.sanger.ac.uk/cosmic). Shown are schematic representations of the lungs with their lobes (RUL, right upper lobe; RML, right middle lobe; RLL, right lower lobe; LUL, left upper lobe; LLL, left lower lobe) and the number (*n*) and percentage of tumors observed. Color indicates frequency. (J) Crosstabulations of relapse events in the GLAD by cTNM7 stage. Shown are patient numbers (*n*), color-coded frequencies by age grouping, and Fisher's exact probabilities (*P*)

lepidic-predominant tumors in LADC was more frequent in never-smokers and displayed lower overall TNM descriptors, decreased metastatic propensity, and prolonged overall survival, as opposed to solid-predominant LADC that displayed aggressive features and poor survival (Figure S8), further validating GLAD.

3.10 | Patterns of relapse

Over 704 cumulative follow-up years, 190 relapse events were identified in 120 patients (Figure 1D). In addition to the associations described above, patients with higher cTNM7 descriptors had higher relapse rates, both at the

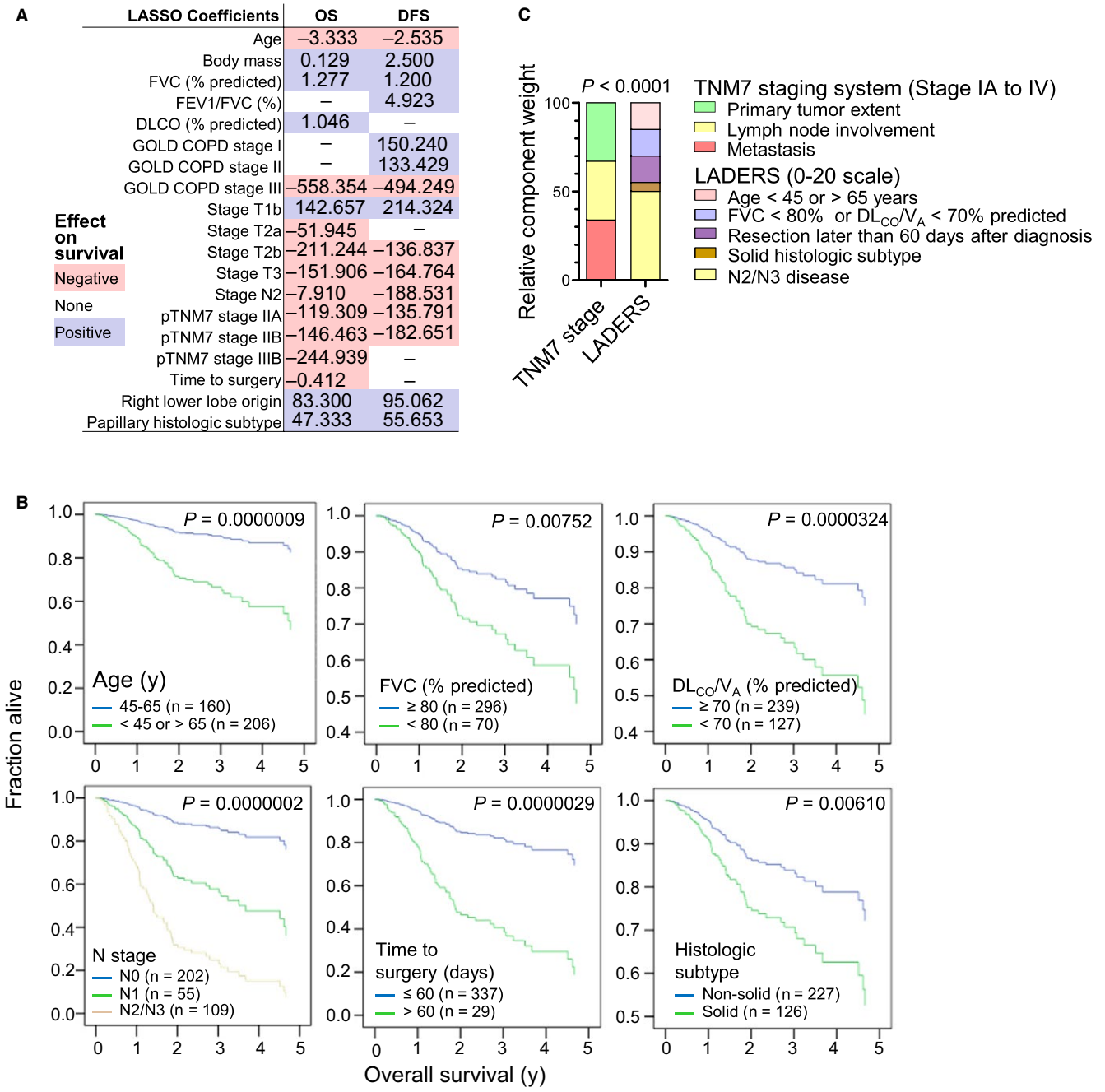


FIGURE 5 Development of the locoregional lung adenocarcinoma death risk score (LADERS) from the GLAD derivation cohort. (A) Results of least absolute shrinkage and selection operator (LASSO) regression. Shown are regression coefficients for overall (OS) and disease-free (DFS) survival and color-coded direction of impact on survival. (B) Results of Cox regression showing proportional hazards survival plots for the six independent predictors of survival of GLAD, including sample sizes (*n*) and probability values (*P*). (C) Schematic representation of the components and relative weight of the variables that comprise LADERS compared with the TNM staging system, including χ^2 probability value (*P*). FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 seconds; DL_{CO}, uncorrected lung diffusion capacity for carbon monoxide; V_A, alveolar ventilation; GOLD, global initiative for chronic obstructive lung disease; COPD, chronic obstructive pulmonary disease; TNM, tumor-node-metastasis staging system; p, pathologic

30-day postsurgery and at mid-2016 benchmarks (Figures 4J and 5). Relapse timing and site did not significantly impact OS; however, pleural or multi-site relapse (5/20 patients with multiple relapses also had pleural relapse) adversely impacted DFS indicating that pleural relapse occurs earlier than others (Figure S9).

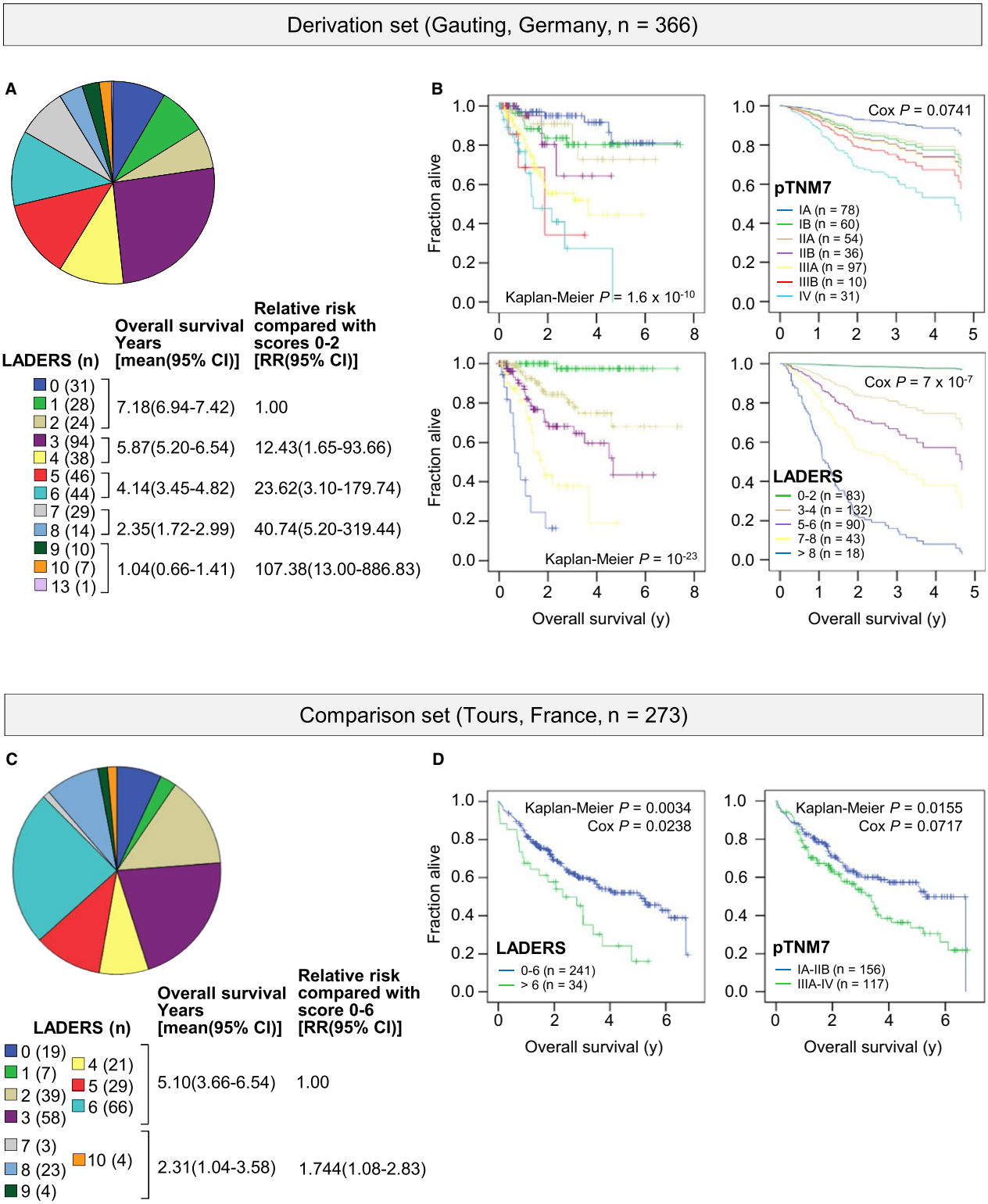


FIGURE 6 Performance of LADERS and pTNM7 as prognosticators in two locoregional lung adenocarcinoma patient cohorts. (A, B) Results from the GLAD derivation cohort. (C, D) Results from the Tours validation cohort. (A, C) Shown are LADERS distribution pie charts and patient numbers (*n*), LADERS groupings employed, and mean (because median was not reached for low LADERS scores) Kaplan-Meier survival and Cox proportional hazards estimates with 95% confidence intervals (95%CI) of LADERS groupings. (B, D) Shown are Kaplan-Meier and Cox proportional hazards survival plots and overall log-rank test and Cox probability values (*P*) for LADERS and pTNM7 groupings, showing that LADERS more accurately predicted death events. TNM, tumor-node-metastasis staging system; p, pathologic; LADERS, locoregional lung adenocarcinoma death risk score

3.11 | Survival

We next assessed the impact of each variable on OS and DFS. In a first step, Kaplan-Meier analyses using OS and DFS as target and single variables as inputs (continuous numerical variables were dichotomized at abnormal cutoffs) showed that patients with age outlying 45-65 years, abnormal percentage predicted FVC and DL_{CO}/V_A, high T, N, and cTNM7 descriptors, delayed and incomplete resection, solid histologic subtype, and pleural relapse; had decreased OS and/or DFS (Figures 4A and 4F, Figures S5E and F, S6, S8C and D, S9B). All variables were entered into a second line least absolute shrinkage and selection operator (LASSO) regression analysis that identified age, body mass, percentage predicted FVC, FEV_1/FVC ratio, percentage predicted DL_{CO}, GOLD COPD stage, T, N, and cTNM7 stage, time to surgery, right lower lobe origin, and histologic subtype as determinants of OS and/or DFS (Figure 5A). In a final step, all variables that emerged both from Kaplan-Meier and LASSO analyses were entered into Cox regression using backward Waldman elimination, which identified age outlying 45-65 years, abnormal percentage predicted FVC and DL_{CO}/V_A, N2/3 disease, delayed resection, and solid histology as independent predictors of OS of GLAD (Figure 5B).

3.12 | The locoregional lung adenocarcinoma death risk score (LADERS)

We next built a model to predict OS at the 30-day postresection benchmark, using the six variables that withstood Kaplan-Meier, LASSO, and Cox regression testing using OS as the target. LADERS employs Cox proportional hazard points and was tailored for easy clinical use without extra imaging/procedures (Figures 5C and 6A and B, Table 1). LADERS displayed only 25% correlation with pTNM7 and was intimately linked with death events, while pTNM7 showed tight linkage with relapse events (Figure S10A and B). LADERS outperformed pTNM7 in predicting OS of GLAD in Kaplan-Meier and Cox regression analyses, while pTNM7 performed better in predicting DFS (Figure S10C; Table 2). LADERS also outperformed pTNM7 in predicting OS in the Tours cohort (Figure 6C and D).

4 | DISCUSSION

Here we present GLAD, a prospectively evolving biobank of phenotypes and tumor/normal paired tissues of patients with locoregional LADC. The longitudinal follow-up of the cohort suggests that locoregional LADC is currently a chronic lung disease with median survival >7.5 years. We corroborate pertinent findings of previous studies, such as the high frequency of these tumors in never/ex-smokers and women and the significant overlap of LADC with COPD, the upper lobe predominance of these tumors that appears to be dexterous in smokers, as well as the value of current staging and histologic typing systems in management and prognosis.[1,2,22] Using detailed phenotyping and prolonged follow-up, we discovered previously ill-defined and undefined clinical associations, such as the adverse effects of extreme age, poor lung function, and delayed resection on survival, as well as the early nature of pleural and the latency of pulmonary relapse. Most of our findings are corroborated in a separate patient cohort from France. Most importantly, we combined this wealth of clinical information to produce LADERS, a clinical score that accurately predicts survival in both cohorts.

TABLE 1 Independent predictors of survival identified by proportional hazards Cox regression analysis and locoregional lung adenocarcinoma death risk score (LADERS)

Variable	Hazard ratio (95% confidence interval)	Probability	Hazard points
Age <45 or >65 years	4.12 (2.35-7.23)	0.0000008	3
FVC[a] <80% predicted	2.13 (1.25-3.63)	0.0054374	1
DL_{CO}/V_A[b] <70% predicted	2.62 (1.60-4.29)	0.0001292	2
N2[c]	3.56 (2.20-5.76)	0.0000002	2.5
N3[c]	8.65 (1.10-68.21)	0.0406576	7.5
Time to surgery >60 days[d]	4.04 (2.07-7.88)	0.0000408	3
Solid histologic subtype[e]	2.09 (1.27-3.43)	0.0035422	1
LADERS[f]			0-20

[a]FVC, forced vital capacity. Compared with FVC ≥80% predicted. When FVC not available, GOLD COPD ≥stage II was used in the Tours cohort.
[b]DL_{CO}/V_A, Lung diffusion capacity for carbon monoxide corrected for alveolar ventilation. Compared with DL_{CO}/V_A ≥70% predicted. When DL_{CO}/V_A not available, current smoking was used in the Tours cohort.
[c]N, cTNM7 nodal status descriptors. Compared with pooled patients with N0 and N1.
[d]Compared with patients operated within 60 days from diagnosis.
[e]Compared with all other histologic subtypes combined, including lepidic, acinar, papillary, micropapillary, adenosquamous, fetal, and non-specified.
[f]Rounded to the lower integer when decimal.

TABLE 2 Performance of pTNM7 and LADERS scores as predictors of survival of GLAD patients at discharge from thoracic surgery (30 days postsurgery census)

		Cox regression[c]						Survival[d]		
Score[a]	Outcome[b]	ND	DEV	DOF	AIC	CON	R^2	iAUC	B-score	B vs KM
pTNM7	OS	775.33	725.27	6	737.3	0.729	0.128	0.676	0.130	0.301
LADERS	OS	775.33	**671.58**	11	**693.6**	**0.804**	**0.247**	**0.764**	**0.118**	**0.366**
pTNM7	DFS	1727.57	**1569.58**	6	**1581.6**	**0.770**	**0.351**	**0.709**	**0.156**	**0.310**
LADERS	DFS	1727.57	1645.75	11	1667.8	0.701	0.200	0.672	0.182	0.195

The bold values highlight the outperformance of LADER or pTNM7 in the different tests.

[a]pTNM7, pathologic tumor-node-metastasis staging system, 7th edition; LADERS, lung adenocarcinoma death risk score.

[b]OS, overall survival; DFS, disease-free survival.

[c]Cox regression parameters: ND, null deviance; DEV, deviance; DOF, degrees of freedom; AIC, area in the curve; CON, concordance; R^2, Pearson's correlation coefficient.

[d]Survival parameters: iAUC, integral area under the curve; B-score, Brier' concordance score; B vs KM, Brier skill vs Kaplan-Meier.

In accord with only one previous report,[28] young GLAD patients developed more aggressive LADC, possibly attributable to germline tumor suppressor loss, a hypothesis that can be directly tested in GLAD tissues. On the other hand, extremely old patients appeared to have a worse surgical prognosis associated with reduced lung and overall function. Active smokers had low N stage and relapse rates, findings possibly related with the young age and increased surveillance of active smokers in our cohort.

For the first time, we report how important time to surgery is for incipient survival, underscoring the aggressiveness of the disease and the urgency of surgery. We also define a previously reported spatial pattern of LADC development in the upper lobes.[29] Although the clinical importance of this finding is unclear, it is likely the result of increased local conversion of inhaled precarcinogens to active carcinogens in the upper lobes of smokers. Of special note, we identify distinct temporal trends in organ-specific relapse of early-stage LADC, similar to biphasic metastatic patterns of other tumor types like breast cancer.[30] Importantly, we provide clinicians with LADERS, an easy-to-use and accurate clinical tool to predict survival.

In conclusion, the first results from a prospective cohort of patients with locoregional LADC corroborate the impact of current staging and histologic subtyping systems and identify important effects of age, lung function, and time to resection on survival. A clinical tool to assess survival is also provided. Importantly, future combination of clinical information with tissue profiling is anticipated to unveil novel tumor genome-phenome links and unprecedented mechanistic insights into evolution of carcinogenesis in the respiratory tract.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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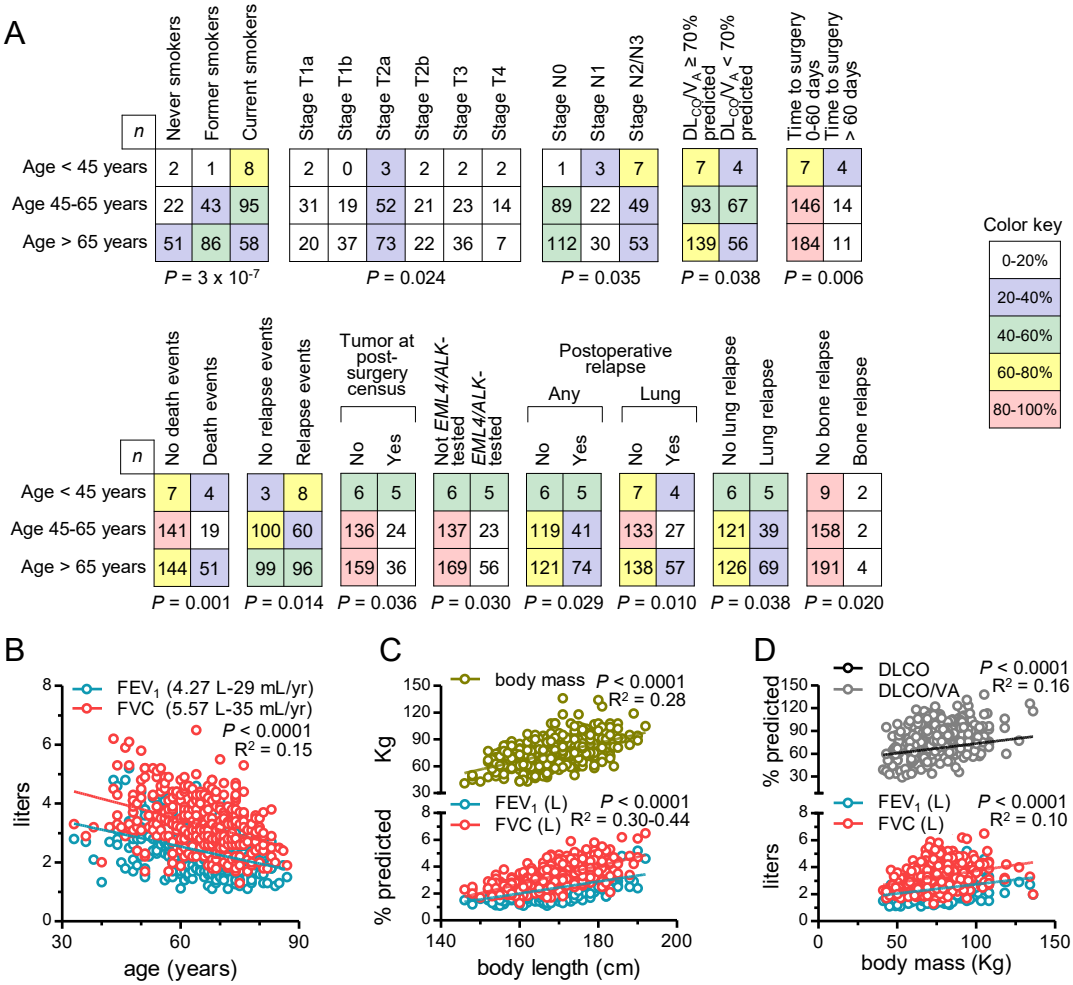


Figure S1. Impact of age on GLAD.

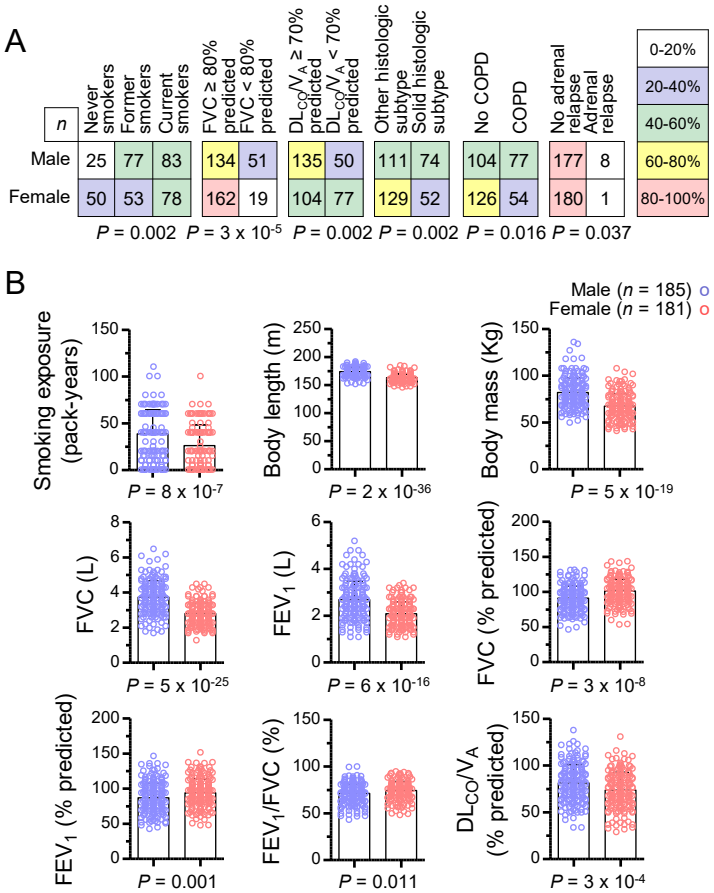


Figure S2. Associations with sex in the GLAD cohort.

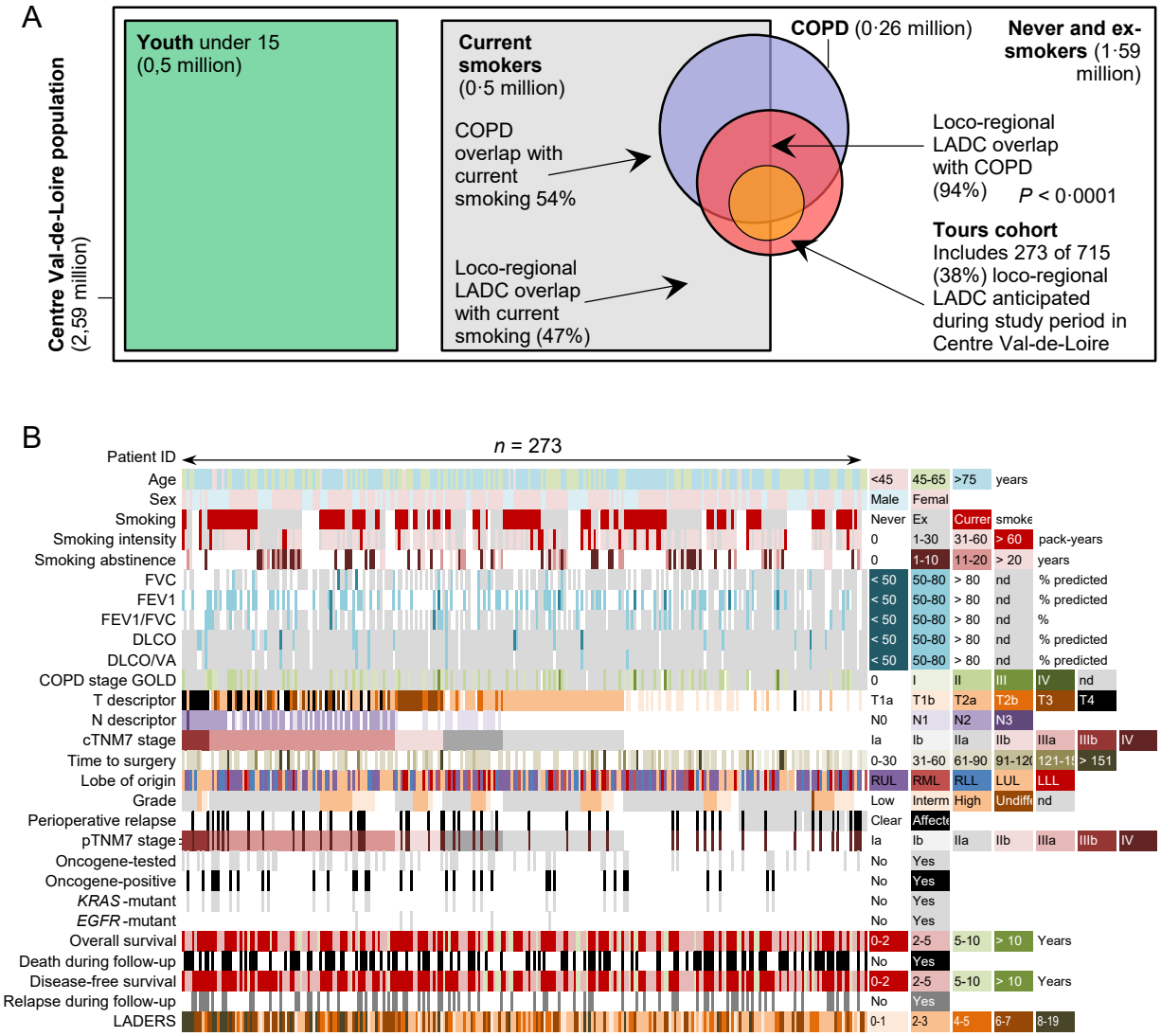


Figure S3. The Tours locoregional lung adenocarcinoma donors cohort.

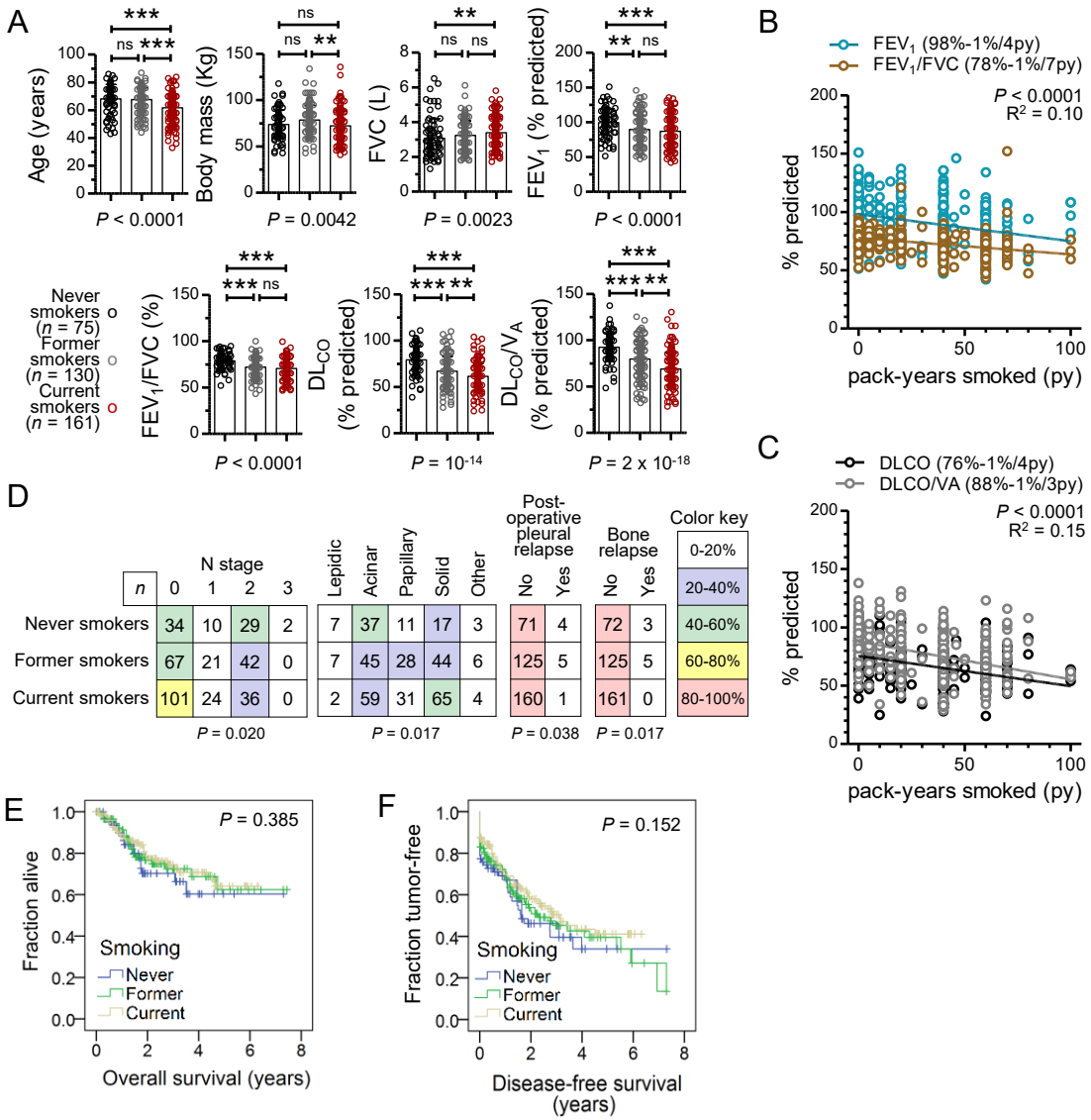


Figure S4. Smoking status, exposure, and effects in the GLAD cohort.

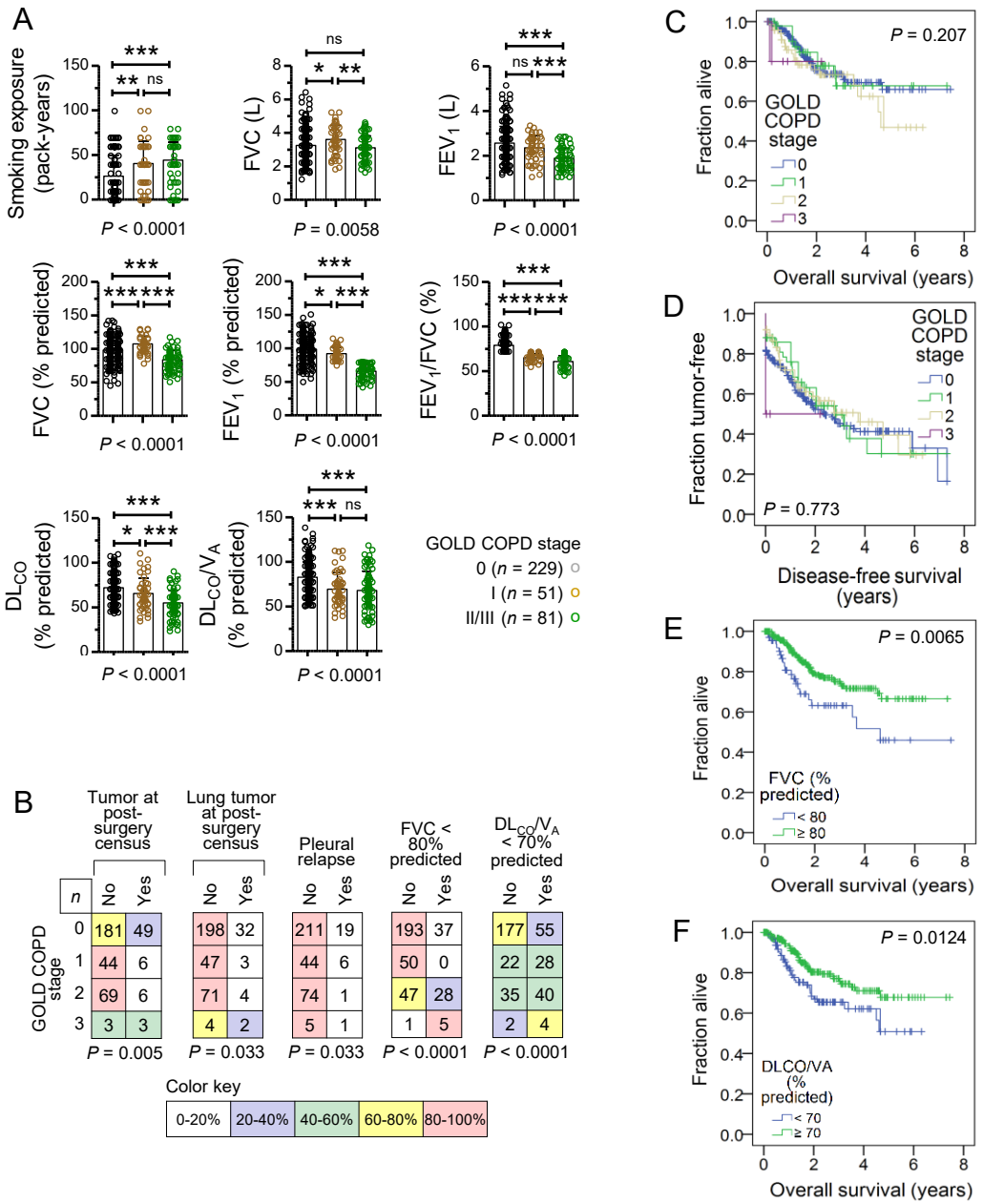


Figure S5. Chronic obstructive pulmonary disease (COPD) in the GLAD cohort.

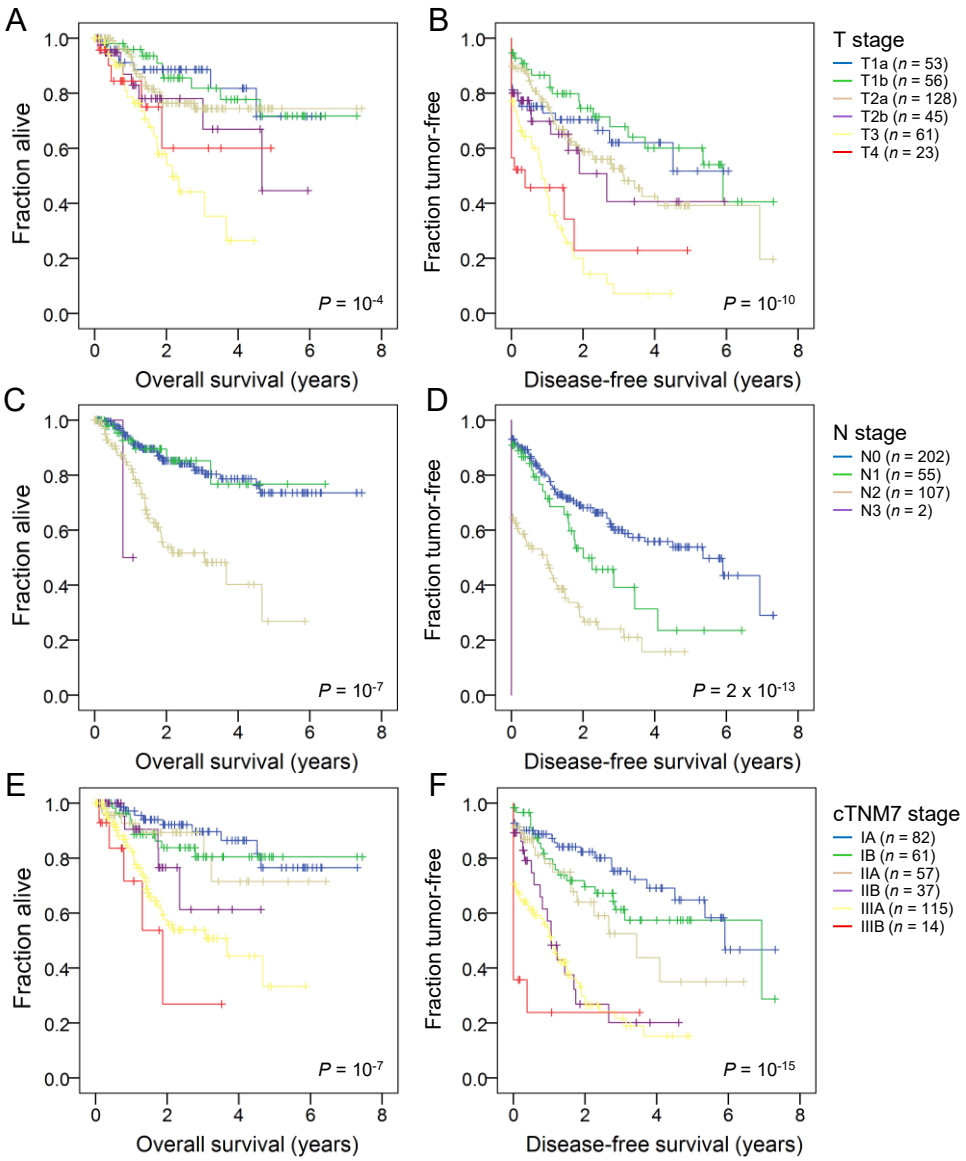


Figure S6. Validation of GLAD cTNM7 staging.

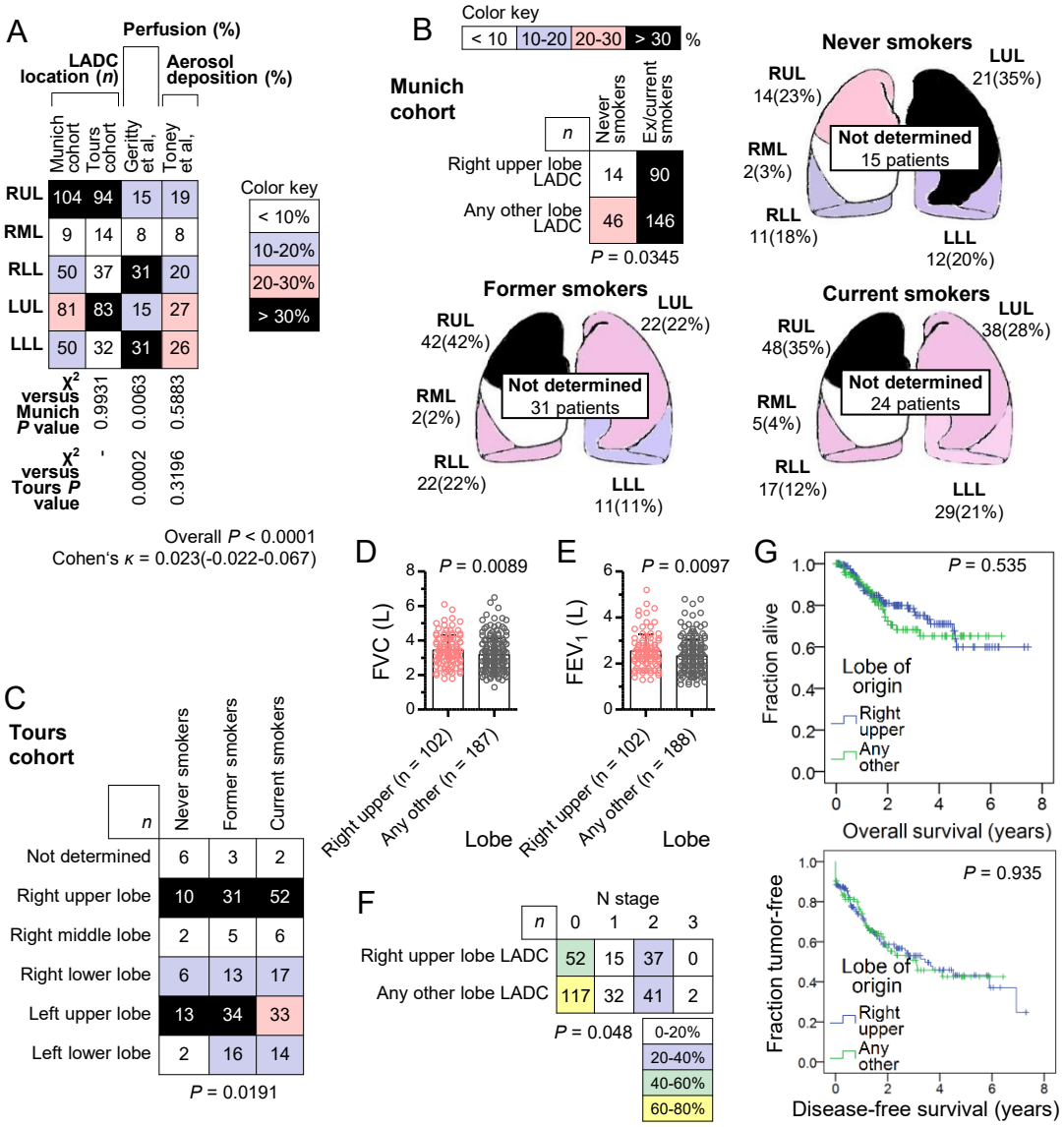


Figure S7. Tumor location on the GLAD and Tours cohorts.

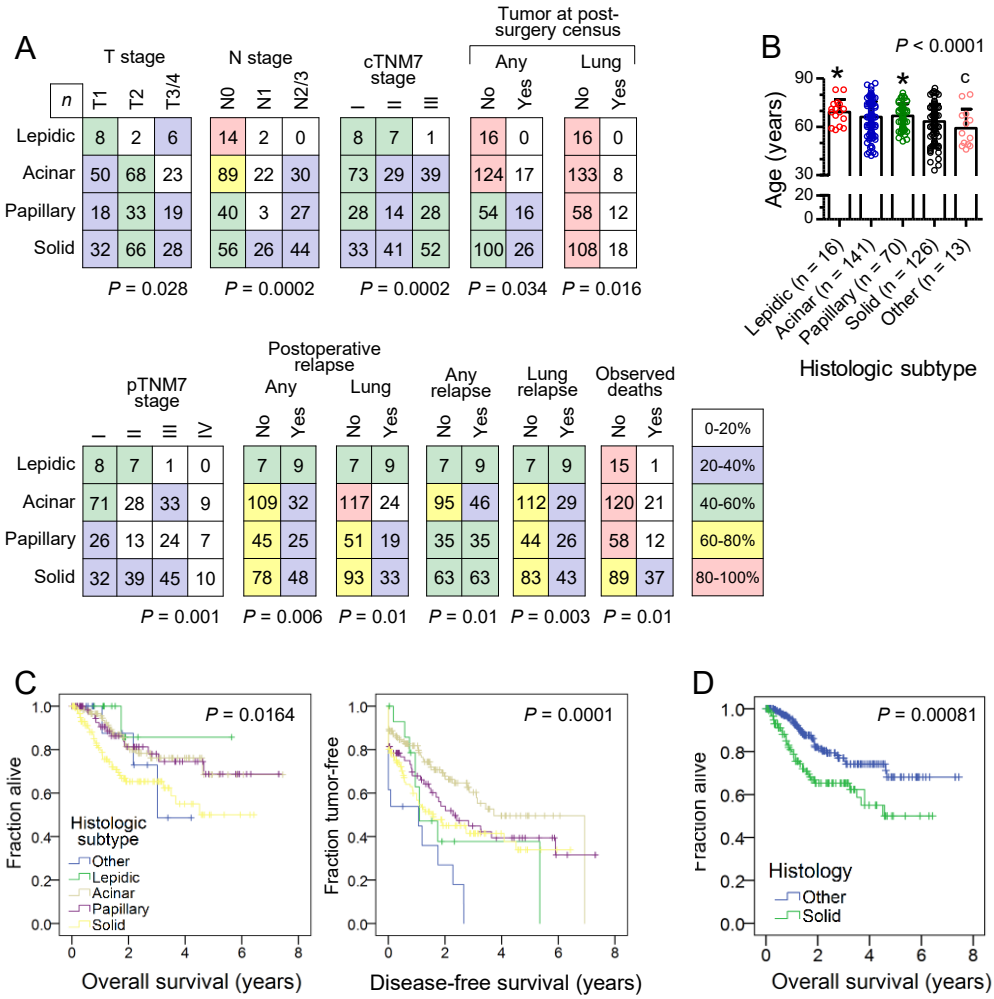


Figure S8. Impact of histologic subtype on outcomes in the GLAD cohort.

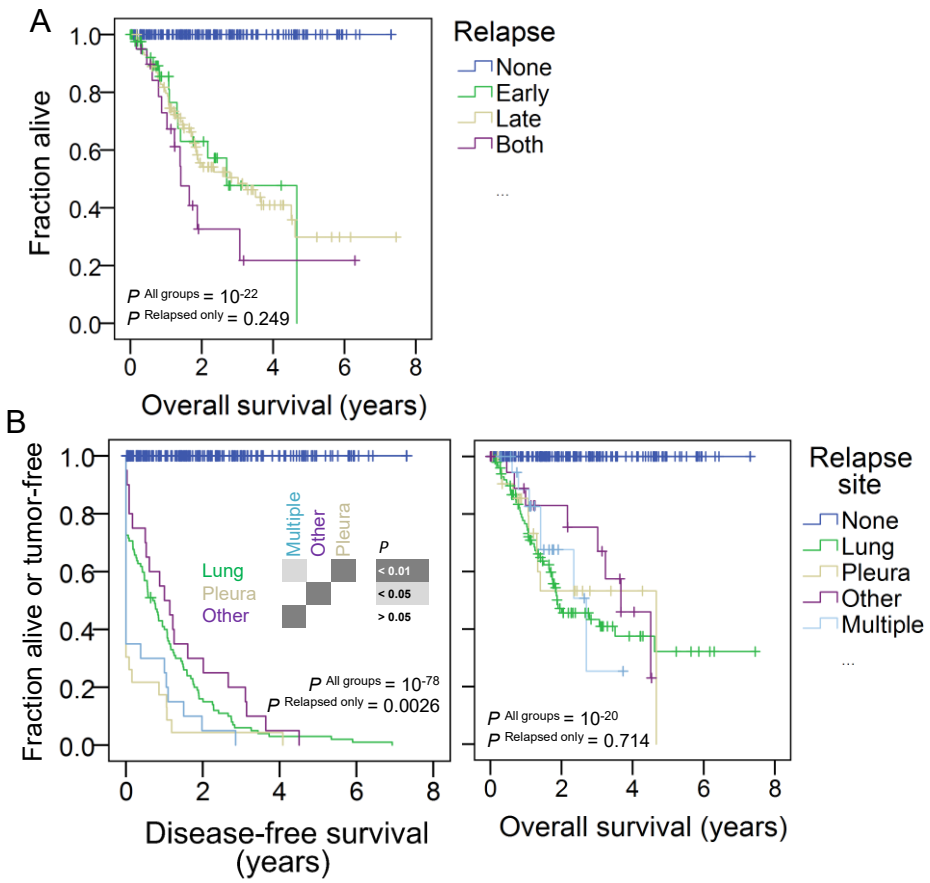
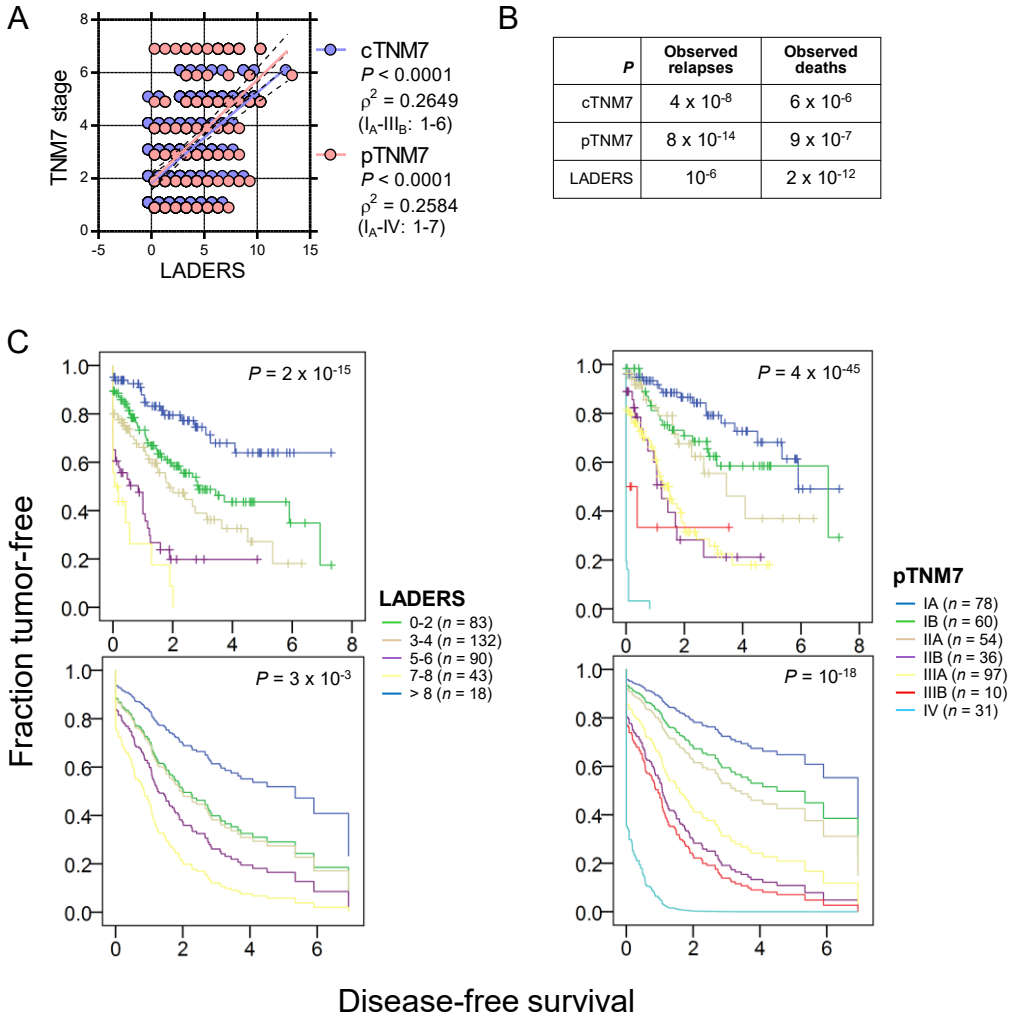


Figure S9. Patterns of relapse of GLAD..



P	Observed relapses	Observed deaths
cTNM7	4×10^{-8}	6×10^{-6}
pTNM7	8×10^{-14}	9×10^{-7}
LADERS	10^{-6}	2×10^{-12}

Figure S10. Comparison of the lung adenocarcinoma death risk score (LADERS) to TNM systems and their impact on disease-free survival.

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Impact of age on GLAD. (A) Crosstabulations of GLAD by age groupings. Shown are patient numbers (*n*), color coded frequencies by age grouping, and Fischer's exact probabilities (*P*). **(B-D)** Correlations of age and body metric indices with lung function tests (*n* = 366). Shown are raw data points (dots), linear regression lines and coefficients, and squared Pearson's correlation coefficients (R^2) and probabilities (*P*). *n*, sample size; DL_{CO}, uncorrected lung diffusion capacity for carbon monoxide; V_A, alveolar ventilation; *EML4*, echinoderm microtubule associated protein like 4; *ALK*, anaplastic lymphoma kinase; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 sec.

Figure S2. Associations with sex in the GLAD cohort. **(A)** Crosstabulations of GLAD by sex. Shown are patient numbers (*n*), color coded frequencies by sex, and Fischer's exact probabilities (*P*). **(B)** Smoking exposure, body metric indices, and lung function parameters stratified by sex. Shown are patient numbers (*n*), raw data points (dots), mean (columns), SD (bars), and Mann Whitney test probabilities (*P*).

Figure S3. The Tours locoregional lung adenocarcinoma donors cohort. **(A)** Venn diagram of current smoking COPD prevalence, and LADC incidence over the Tours study. **(B)** Phenome plot of the Tours cohort (raw data available in Table S2). Color-coded pivot table of all data obtained sorted sequentially by cTNM7 stage, tumor grade, sex, and smoking status. Columns represent individual patients and rows variables recorded. *n*, sample size; ID, identifier; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 sec; DL_{CO}, uncorrected lung diffusion capacity for carbon monoxide; V_A, alveolar ventilation; COPD, chronic obstructive pulmonary disease; GOLD, global initiative for chronic obstructive lung disease; TNM, tumor-node-metastasis staging system; c, clinical; p, pathologic; *EGFR*, epidermal growth factor receptor; *KRAS,* V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; LADERS, locoregional lung adenocarcinoma death risk score; nd, not determined.

Figure S4. Smoking status, exposure, and effects in the GLAD cohort. **(A)** Age, body mass, and lung function parameters stratified by smoking status. Shown are patient numbers (*n*), raw data points (dots), mean (columns), SD (bars), and Kruskal-Wallis test probabilities (*P*). ns, **, and ***: $P > 0.05$, $P < 0.01$, and $P < 0.001$, respectively, for the indicated comparisons by Dunn's post-tests. **(B, C)** Correlations of smoking exposure with lung function tests (*n* = 366). Shown are raw data points (dots), linear regression lines and coefficients, and squared Pearson's correlation coefficients (R^2)

and probabilities (*P*). **(D)** Crosstabulations of GLAD by smoking status. Shown are patient numbers (*n*), color coded frequencies by smoking status, and Fischer's exact probabilities (*P*). **(E, F)** Kaplan-Meier disease-free and overall survival plots and overall log-rank test probability values (*P*) of the GLAD stratified by smoking status (*n* = 75, 130, and 161, respectively, for never, former, and current smokers). *n*, sample size; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 sec; DL_{CO}, uncorrected lung diffusion capacity for carbon monoxide; V_A, alveolar ventilation.

Figure S5. Chronic obstructive pulmonary disease (COPD) in the GLAD cohort. **(A)** Age and lung function parameters stratified by COPD stage (*n* = 361 due to missing data in five patients) as defined by the global initiative for chronic obstructive lung disease 2001 criteria [18]. Shown are patient numbers (*n*). raw data points (dots), mean (columns), SD (bars), and Kruskal-Wallis test probabilities (*P*). ns, *, **, and ***: $P > 0.05$, $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively, for the indicated comparisons by Dunn's post-tests. **(B)** Crosstabulations of GLAD by GOLD COPD stage. Shown are patient numbers (*n*), color coded frequencies by age grouping, and χ^2 probabilities (*P*). **(C, D)** Kaplan-Meier disease-free and overall survival plots and overall log-rank test probability values (*P*) of the GLAD stratified by GOLD COPD stage (*n* = 229, 51, 75, and 6, respectively, for GOLD COPD stages 0, I, II, and III). **(E, F)** Kaplan-Meier overall survival plots and log-rank test probability values (*P*) of the GLAD stratified by normal or abnormal forced vital capacity (FVC) and lung diffusion capacity for carbon monoxide corrected for alveolar ventilation (DL_{CO}/V_A) (FVC: *n* = 296 and 70, respectively, for values ≥ 80% and < 80%; DL_{CO}/V_A: *n* = 239 and 127, respectively, for values ≥ 70% and < 70%). *n*, sample size; FEV_1, forced expiratory volume in 1 sec; DL_{CO}, uncorrected lung diffusion capacity for carbon monoxide.

Figure S6. Validation of GLAD cTNM7 staging. Kaplan-Meier overall **(A, C, E)** and disease-free **(B, D, F)** survival plots and overall log-rank test probability values (*P*) of the GLAD stratified by T (A, B), N (C, D), and cTNM7 (E, F) stage. *n*, sample size; TNM, tumor-node-metastasis staging system; c, clinical.

Figure S7. Tumor location in the GLAD and Tours cohorts. **(A)** Crosstabulation of LADC location by lung lobe in the GLAD and Tours cohorts with lobar ventilation patterns determined by inhaled particle deposition and lobar perfusion patterns assessed via injected radioisotope distribution. Shown are number (*n*) of LADC observed, percentage of inhaled or injected particle distribution, overall χ^2 probability (*P*) value, χ^2 probability (*P*) values for comparison of each study to GLAD and Tours cohorts, and overall Cohen's κ coefficient of agreement. Color indicates frequency. **(B, C)** LADC location by lung lobe determined at surgery in the GLAD derivation cohort and a smoking-optimal

comparison cohort from Tours, France that is presented in detail in Supplementary Table 2 and Figure 3. Shown are schematic representations and crosstabulations of the lungs with their five lobes (RUL, right upper lobe; RML, right middle lobe; RLL, right lower lobe; LUL, left upper lobe; LLL, left lower lobe) and the number (*n*) and percentage of tumors observed in never, former, and current smokers, and Fischer's exact (B) or χ^2 (C) probabilities (*P*). Color indicates frequency. **(D, E)** Selected lung function parameters stratified by lobar tumor location in the GLAD cohort. Shown are patient numbers (*n*), raw data points (dots), mean (columns), SD (bars), and Student's t test probabilities (*P*). **(F)** Crosstabulation of tumor location by N stage in GLAD. Shown are patient numbers (*n*), color coded frequencies by age grouping, and Fischer's exact probability (*P*). **(G)** Kaplan-Meier disease-free and overall survival plots and log-rank test probability values (*P*) of GLAD patients stratified by tumor location in the right upper (*n* = 104) or any other (*n* = 29) lung lobe. *n*, sample size; LADC, lung adenocarcinoma; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 sec.

Figure S8. Impact of histologic subtype on outcomes in the GLAD cohort. **(A)** Crosstabulations of histologic subtype by stage, relapse, and death related variables. Shown are patient numbers (*n*), color coded frequencies by histologic subtype, and Fischer's exact probabilities (*P*). **(B)** Age stratified by histologic subtype. Shown are patient numbers (*n*), raw data points (dots), mean (columns), SD (bars), and Kruskal-Wallis test probability (*P*). *: $P < 0.05$ for comparison to other histologic subtype control (c) by Dunn's post-tests. **(C)** Kaplan-Meier disease-free and overall survival plots and overall log-rank test probability values (*P*) of GLAD patients stratified by distinct histologic subtypes (*n* = 13, 16, 141, 70, and 126, respectively, for other, lepidic, acinar, papillary, and solid subtypes). **(D)** Kaplan-Meier overall survival plot and log-rank test probability value (*P*) of GLAD patients classified into solid (*n* = 126) and other non-solid (*n* = 240) histologic subtypes. *n*, sample size; TNM, tumor-node-metastasis staging system; c, clinical; p, pathologic.

Figure S9. Patterns of relapse of GLAD. (A) Kaplan-Meier overall survival plot and overall log-rank test probabilities inclusive ($P^{\text{All groups}}$) and non-inclusive ($P^{\text{Relapsed only}}$) of patients without relapse of GLAD patients stratified by timing of relapse: no relapse (*n* = 201), early relapse (prior to the 30-day post-resection census; *n* = 45), late relapse (thereafter; *n* = 100), or both (*n* = 20). **(B)** Kaplan-Meier disease-free and overall survival plots and overall and pairwise (table insert) log-rank test probabilities inclusive ($P^{\text{All groups}}$) and non-inclusive ($P^{\text{Relapsed only}}$) of patients without relapse of GLAD patients stratified by site of relapse: no relapse (*n* = 201), pulmonary relapse (*n* = 102), pleural relapse (*n* = 23), other extrathoracic relapse (*n* = 20), or multiple relapse sites (*n* = 20; five had also pleural relapse). *n*, sample size; TNM, tumor-node-metastasis staging system; c, clinical.

Figure S10. Comparison of the lung adenocarcinoma death risk score (LADERS) to TNM systems and their impact on disease-free survival. (A) Correlations of LADERS with cTNM7 (blue) and pTNM7 (red; *n* = 366 for both). Shown are raw data points (dots), linear regression lines with 95% confidence intervals, and squared Pearson's correlation coefficients (R^2) and probabilities (*P*). **(B)** χ^2 probability (*P*) values for crosstabulations of cTNM7, pTNM7, and LADERS with observed relapses and deaths in the GLAD (shown in B) show pTNM7 to be closest linked with relapse, but LADERS with death events. **(C)** Kaplan-Meier (top) and Cox proportional hazards (bottom) disease-free survival plots and overall log-rank test and Cox probability values (*P*) for LADERS and pTNM7 groupings confirm the closer linkage of pTNM7 to relapse events. *n*, disease; TNM, tumor-node-metastasis staging system; c, clinical; p, pathologic.

PUBLICATION II

Prognostic relevance of regional lymph-node distribution in patients with N1-positive non-small cell lung cancer: A retrospective single-center analysis.

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Prognostic relevance of regional lymph-node distribution in patients with N1-positive non-small cell lung cancer: A retrospective single-center analysis

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ABSTRACT

Objective: Lymph node (LN) metastases predict survival in patients with non-small cell lung cancer (NSCLC) treated with curative surgery. Nevertheless, prognostic differences within the same nodal (N) status have been reported. Consequently, the International Association for the Study of Lung Cancer (IASLC) proposed to stratify patients with limited nodal disease (pN1) from low (pN1a) to high (pN1b) nodal tumor burden. This study aimed to validate the IASLC proposal in a large single-center surgical cohort of patients with pN1 NSCLC.

Material and Methods: Data from 317 patients with pN1 NSCLC treated between January 2012 and December 2016, were retrospectively analyzed. Associations between distribution of LN metastases and survival were analyzed for different classification models—toward nodal extension (pN1a: one station involved; pN1b: multiple stations involved) and toward location (pN1 in the hilar [LN#10/11] or peripheral zone [LN#12-14]).

Results: Tumor-specific survival (TSS) in the entire pN1 cohort was 67.1% at five years. Five-year TSS rates for pN1a and pN1b patients were comparable (67.6% vs. 66.5%, $p = 0.623$). Significant survival differences from pN1a to pN1b were observed only in patients with adenocarcinoma histology and completed adjuvant chemotherapy (5-year TSS: pN1a, 80.4% vs. pN1b, 49.6%; $p = 0.005$). TSS for LN metastases in the hilar zone/peripheral zone or in both zones was 68.2% and 59.9%, respectively ($p = 0.068$). In multivariate analysis, adjuvant chemotherapy, squamous cell histology, and nodal disease limited to one zone nodal disease were identified as independent beneficial prognostic factors ($p < 0.05$).

Conclusion: pN1 in only one region (hilar or lobar) was associated with better outcome than metastatic affection of both regions after surgery and adjuvant therapy. A stratification towards single (pN1a) and multiple (pN1b) N1-metastases was found of prognostic relevance only in adenocarcinoma. Prospective multicenter analysis of prognostic subgroups in N1 NSCLC is required to evaluate its clinical impact for consideration in future TNM classification.

1. Introduction

Lung cancer is one of the leading causes of death worldwide [1]. Approximately 80% of lung cancer patients are consistent with non-small cell histology (NSCLC) [2]. The Tumor-Node-Metastasis (TNM) system is used to classify and stratify patients by prognosis according to tumor size (T), lymph node (LN) involvement (N) and presence of distant metastases (M). Its actual valid 8th edition was implemented in 2016 using clinical information from 70,976 consecutive NSCLC patients treated between 1999 and 2010 in 16 countries [3]. In patients with limited thoracic disease (non-stage IV), nodal metastases have been repeatedly identified as a strong prognostic predictor [4,5]. The N descriptor describes the extent of LN metastases and comprises three categories: N1 (ipsilateral hilar or ipsilateral lobar LN metastases); N2 (ipsilateral mediastinal LN metastases; subcarinal LN metastases); and N3 (contralateral hilar or mediastinal LN metastases; supraclavicular LN metastases).

For N1-positive NSCLC, radical surgical treatment followed by adjuvant chemotherapy is recommended [6–9]. Tumor resection (at least lobectomy) is accompanied by radical LN compartment dissection to enable precise nodal staging and to plan stage-dependent adjuvant treatment, thus improving survival [10–12]. Resected intra-thoracic LNs are consecutively numbered following a commonly used LN mapping system that was introduced by Mountain and Dresler (M-D map) in 1997 and modified in 2009 [13].

To analyze possible prognostic differences within the same N1

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category, the IASLC nodal map distinguishes N1 nodes more precisely into a hilar/interlobar zone (M-D map: levels 10/11), and a peripheral zone (M-D map levels 12–14) [14]. The clinical relevance of further sub-classification has been noted by several authors who found a worsening prognosis from peripheral N1 (levels 12–14) to more centrally affected N1 LN metastases (levels 10/11) [15,16].

The prognostic value of a more detailed subclassification of the N1 descriptor has been recently discussed by the IASLC in a proposal addressing the implementation of the revised 8th TNM-edition for NSCLC. The adjusted pN1 cohort from the IASLC database comprises data from 3554 patients with completely resected NSCLC. A better outcome was demonstrated for patients with N1 metastases in only a single level (pN1a) compared with multiple-level N1 (pN1b) [17]. No precise information is available regarding histology, extent of tumor resection (lobar/sublobar), and adjuvant (chemo) therapy. Moreover, European data (applicable data only from Norway and Serbia) accounts for only 15% of all analyzed N1 patients (n = 544).

The aim of our study, therefore, was to validate the different N1 subclassification systems proposed by the IASLC in a large European cohort of pN1 patients. All patients underwent curative intent lung cancer surgery in a single high-volume thoracic center in Germany (Thoraxklinik Heidelberg, Heidelberg University, Heidelberg, Germany). Associations between the distribution of pN1 LN-metastases and survival were analyzed and compared with existing data.

2. Material and methods

Data from 484 consecutive surgical patients with pN1 NSCLC, who were treated at the authors' institution between January 2010 and December 2016, were retrospectively reviewed. Patients treated with neoadjuvant therapy and those who underwent only sublobar or incomplete tumor resections were excluded. Furthermore, patients with a histology other than adenocarcinoma or squamous-cell carcinoma, and those who died independent of tumor progression within 90 days after surgery were also excluded. Ultimately, 317 patients were eligible for further analysis.

Preoperative standard workup in all patients included cardiopulmonary function tests according to individual risk profiles. All patients underwent rigid tracheobronchoscopy for endoscopic evaluation. Distant metastases were ruled out using abdominal, bone, and brain sectional imaging, and, in more recent cases by PET-CT. Endobronchial ultrasound and transbronchial needle aspiration were routinely used for invasive mediastinal nodal staging. Inconclusive results (especially in N2 compartment) were reevaluated by surgical standard mediastinoscopy upfront surgery. Tumor staging of all patients was performed following the 7th edition of the TNM-classification system, which was valid during the complete treatment period. All patients were discussed in the authors' multidisciplinary tumor board. Surgical procedures included anatomical resections depending on tumor extent (such as lobectomy, bilobectomy, and pneumonectomy). Systematic radical compartment dissection of mediastinal (N2) and hilar/interlobar LNs (N1) was an integral part of each procedure.

2.1. LN assessment

All dissected intrathoracic LNs were primary allocated to hilar (#10), interlobar (#11), and lobar (#12-14) compartments according to the M—D map (for non-Asian patients) [13]. Retrospectively, metastatic nodes were classified into potential prognostic subgroups:

- Classification by nodal tumor burden: subdivision to pN1a (only one compartment involved) and pN1b (more than one compartment involved) according to the most recent IASLC proposal [17].
- Classification by location of metastatic node: Assignment to hilar zone (nodes #10, #11) and peripheral zone (nodes #12-14) as defined in the IASLC map [14].

2.2. Staging, adjuvant therapy and follow-up

All tumors were classified according to the 7th TNM-edition that was valid at time of diagnosis. Restaging of all tumors towards the recent 8th edition was internally performed in order to evaluate associated changes of prognostic factors. Nevertheless, survival data was calculated following the 7th edition in order to avoid a mismatch between the date of treatment decision and validity of the connected TNM-edition. All patients were discussed in the multidisciplinary tumor board for adjuvant therapy before discharge. According to postoperative recovery, constitution, and risk profile, all patients were recommended to undergo 4 cycles of adjuvant platinum-based doublet chemotherapy. Follow-up visits were scheduled at the outpatient service every 3 months. Recurrence of disease resulted in whole-body restaging and interdisciplinary discussion. Suspicious recurrent lesions were further classified according to their localization (intrathoracic only, distant only, or multiple).

2.3. Survival analysis

Tumor specific survival (TSS) was defined as the time from the date of surgery to the date of tumor-related death or last follow-up in censored, alive patients. Disease-free survival (DFS) was defined as the date of surgery to the date of first detection of recurrence. To analyze prognostic differences within pN1 patients undergoing standard postoperative therapy, univariate survival calculation focused on patients who underwent complete adjuvant chemotherapy.

Data were collected and analyzed using SPSS version 25 (IBM Corporation, Armonk, NY, USA). Cumulative survival was calculated using the Kaplan-Meier product method, while the log rank-test was used to calculate univariate differences, and the Cox-regression model for multivariate analysis. Differences with $p < 0.05$ were considered to be statistically significant.

3. Results

3.1. Patient characteristics

Data from 317 patients (102 females [32.2%]), with a mean (± SD) age of 64.9 ± 9.7 years were retrospectively analyzed. The majority of tumors were upper lobe tumors (n = 191 [60.3%]), solely located on the right (n = 171 [53.9%]). Lobectomies were performed in 207 (65.3%) patients, pneumonectomies in 89 (28.1%), and bilobectomies in 21 (6.6%). Extensions to bronchial sleeve resections were performed in 52 (16.4%) patients. T-stage was pT1 in 32 (10.1%) patients, pT2 in 150 (47.3%), pT3 in 90 (28.4%), and pT4 in 45 (14.2%). Moreover, 182 (57.4%) patients were classified as stages IIA/B and 135 (42.6%) patients were classified as stage IIIA. There were 170 (53.6%) squamous cell carcinomas and 147 (46.4%) adenocarcinomas. The major predominant adenocarcinoma subtypes were as follows: solid (n = 51 [34.7%]), acinar (n = 42 [28.6%]), and papillary (n = 31 [21.1%]) (Table 1).

3.2. LN assessment

At initial clinical staging, 105 patients were classified cN0 and 43 patients were classified cN2. cN1 was stated in 169 patients (53.3%). Thus, nodal upstaging (cN0 but pN1) was observed in 33.1%, downstaging (cN2 but pN1) in 13.6%.

Pathological N1a status (one compartment involved) was found in 207 (65.3%) patients and pN1b (more than one compartment involved) in 110 (34.7%). Of all 207 pN1a patients, 41 (12.9%) exhibited metastases in LN #10, 52 (16.4%) in LN #11, and 114 (36.0%) in LNs #12/13/14. Following IASLC zonal classification, nodal metastases were located exclusively in the hilar (# 10/11) or peripheral zone (#12,13,14) in 93 (29.3%) and 149 (47.0%) patients, respectively. N1

Table 1
Patient characteristics.

	n (%)
No. of patients	**317 (100)**
Gender	
Male	215 (67.8)
Female	102 (32.2)
Age, years: mean (range)	**64.9 (40-89)**
Surgical procedure	
Lobectomy	207 (65.3)
Bilobectomy	21 (6.6)
Pneumonectomy	89 (28.1)
T-Status	
pT1a/b	32 (10.1)
pT2a/b	150 (47.3)
pT3	90 (28.4)
pT4	45 (14.2)
Tumor Stage (UICC 7)	
Stages IIA/IIB	182 (57.4)
Stage IIIA	135 (42.6)
Tumor Stage (UICC 8)	
Stage IIB	138 (43.5)
Stage IIIA	179 (56.6)
Nodal subdivision (stations involved)	
pN1a (one station involved)	207 (65.3)
pN1b (multiple stations involved)	110 (34.7)
Nodal subdivision (distribution to zones)	
Hilar zone only (nodes #10/11)	93 (29.3)
Peripheral zone only (nodes #12-14)	149 (47.0)
Both zones involved	75 (23.7)
Histology	
Squamous cell carcinoma	170 (53.6)
Adenocarcinoma	147 (46.4)
Adjuvant chemotherapy	
Yes	198 (62.4)
No	119 (37.5)
Recurrence at Follow up	
No recurrence	197 (62.1)
Local intrathoracic	31 of 120
Single distant	70 of 120
Multiple	19 of 120
Tumor-specific survival (1-/ 3-/ 5-year)	**90.5%/73.4%/67.1%**
Squamous cell carcinoma	**90.8/75.0%/69.8%**
Adenocarcinoma	**89.4/71.6%/63.9%**
Disease free survival (5-year)	**56.4%**

metastases in both hilar and peripheral zones were found in 75 (23.7%) patients.

3.3. Adjuvant treatment and tumor recurrence

A total of 198 (62.4%) patients underwent adjuvant platinum-based chemotherapy. In 119 (37.5%) patients, adjuvant chemotherapy was not administered. Reasons included comorbidity or questionable evidence with regard to patient age (n = 87 [73.1%]), patient refusal (n = 27 [22.6%]), or progressive disease in the meantime (n = 5 [4.2%]). At the time of last analysis (January 2019), 197 patients (62.1%) were free of recurrence. The calculated DFS at five years was 56.4%. Tumor recurrence was detected in 120 (37.9%) patients, 70 of whom developed distant metastases.

3.4. Long-term survival and prognostic factors

Of the 317 patients, 217 (68.5%) were alive in January 2019 after a median follow up of 35 months. The TSS rate at 5 years was 67.1% (Fig. 1). According to T-category, five-year TSS was 91% for T1, 74% for T2, 53% for T3, and 57% for T4 tumors. These differences were statistically significant (i.e., $p < 0.05$), except for the comparison of T1 and T2 ($p = 0.075$). Comparison of clinical and pathological nodal status revealed no prognostic benefit for incidental finding of N1 (cN0 but pN1 vs. cN1 and pN1, 62.3% vs. 72.1% at five years, $p = 0.277$).

Conclusively, stages IIA/IIB (T1N1/T2N1) were associated with significantly better survival than stage IIIA (T3N1/T4N1) (76.6% vs. 53.8%, respectively; $p = 0.001$) (Fig. 2). Squamous cell carcinoma was associated with a slightly higher survival rate at five years (70%) than adenocarcinoma (64%); however, the difference was not statistically significant ($p = 0.35$). Survival significantly improved in N1 patients who underwent adjuvant chemotherapy (TSS 72% vs. 57% at 5 years; $p = 0.003$).

Comparable survival was observed when all 317 N1 patients were separated into N1a and N1b groups (5-year TSS 67.6% vs. 66.5%; $p = 0.623$).

In univariate subgroup analysis of patients treated with adjuvant chemotherapy, survival differences (comparing pN1a with pN1b) were found only in patients with adenocarcinoma histology (5-year TSS 80.4% [pN1a] vs. 49.6% [pN1b]; $p = 0.005$) (Fig. 3), not in those with squamous cell carcinoma (5-year TSS 69.6% [pN1a] vs. 79.7% [pN1b]; $p = 0.58$). For patients who did not undergo adjuvant therapy, stratification according to the pN1a/pN1b model appeared to have no prognostic impact (adenocarcinoma: 5-year TSS, 47.9% [pN1a] vs. 56.4% [pN1b], $p = 0.82$; squamous cell carcinoma: 5-year TSS, 62.2% [pN1a] vs. 69.1 [pN1b], $p = 0.96$).

Following a location based nodal subgrouping (only hilar zone or peripheral zone), no prognostic difference in between these two groups was observed (74.2% vs. 69.5% at five years $p = 0.849$). Patients with metastases in both the hilar and peripheral zone demonstrated a trend toward poorer survival than those with only one zone affected (hilar or peripheral zone only: 68.2%; both zones: 59.9%, $p = 0.068$) (Fig. 4A). Univariate analysis according to histological subtype revealed that this classification model (toward zones) had a significant influence on survival in patients with adenocarcinoma ($p = 0.001$) but not in those with squamous cell carcinoma ($p = 0.806$) (Fig. 4B and C).

Multivariate analysis of pN1-patients showed that adenocarcinoma histology, high tumor stage, high nodal burden (affection of hilar and peripheral zone) and absence of adjuvant chemotherapy were associated with worse survival compared to the comparators (Table 2). Classification following the pN1a/pN1b model had no significant prognostic impact in multivariate analysis, nevertheless a trend towards better survival for patients with single metastases (pN1a) was observed.

4. Discussion

A total of 317 patients with N1-positive NSCLC, who underwent curative intent surgical therapy, were retrospectively analyzed. Involvement of multiple nodal locations (pN1b) and advanced regional nodal extension (i.e., LN metastases in the central hilar and peripheral zone) were associated with poor prognosis in patients with adenocarcinoma histology.

The individual course of disease in patients with non-metastatic cancer is strongly determined by the presence of LN metastases, reflected in different N descriptors in the TNM-staging system [14]. Nevertheless, prognostic differences have been described within patients stratified according to the same N category [18]. Saji et al. analyzed 689 surgical patients and identified a low number of nodal metastases (1–3 LN metastases) as a beneficial prognostic factor. Moreover, > 4 LN metastases in the N1 compartment was associated with even poorer survival than limited (1–3 LN metastases) pN2-stage. However, only 91 pN1 patients were analyzed [19]. Analysis of the IASLC pN1 cohort also revealed a decrease in survival the more metastatic N1-nodes exist. The authors, therefore, proposed to subdivide pN1 positive NSCLC patients into pN1a (a single station involved, 58% alive at 5 years) and pN1b (multiple stations involved, 50% alive at 5 years) in future TNM-classifications [17]. Analysis of our patients confirmed differences in survival between patients with pN1a and pN1b LN involvement; nevertheless, these were statistically significant only in those with adenocarcinoma histology.

Comparing global data in terms of prognostic relevance of nodal

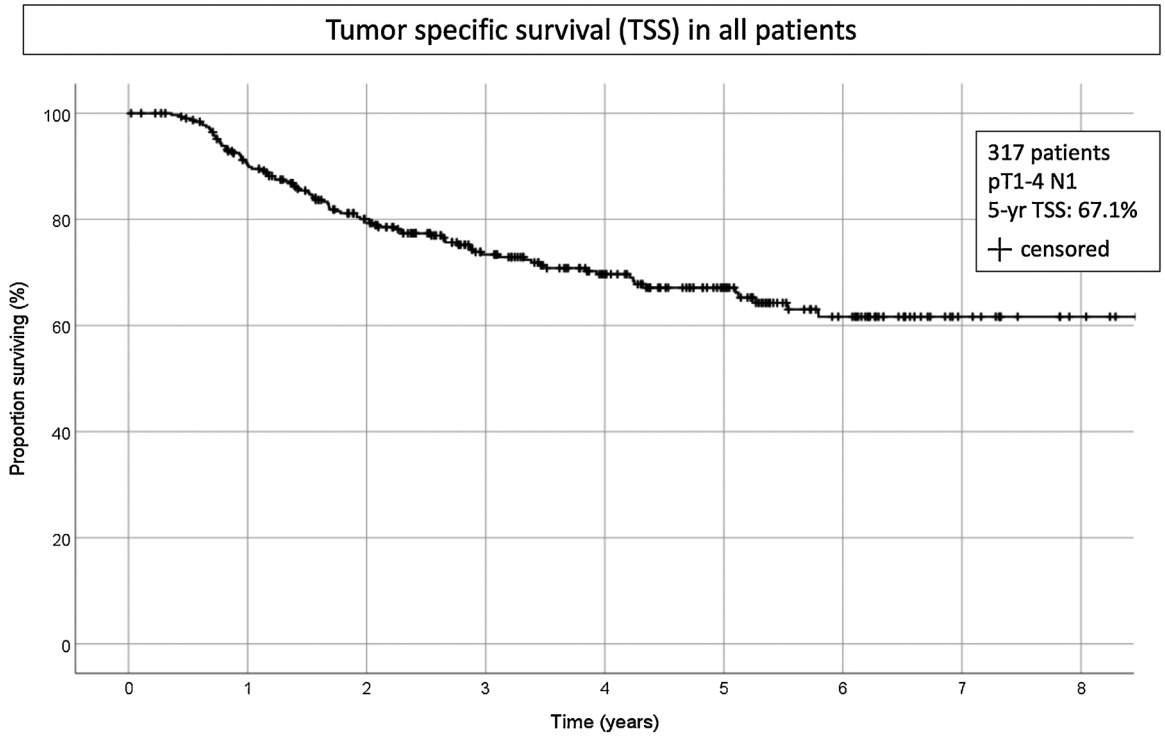

Fig. 1. Tumor specific survival at 5 years was 67.1% for the entire cohort (317 patients, incomplete and sublobar resections excluded; histology other than squamous-cell carcinoma or adenocarcinoma excluded). TSS: Tumor specific survival.

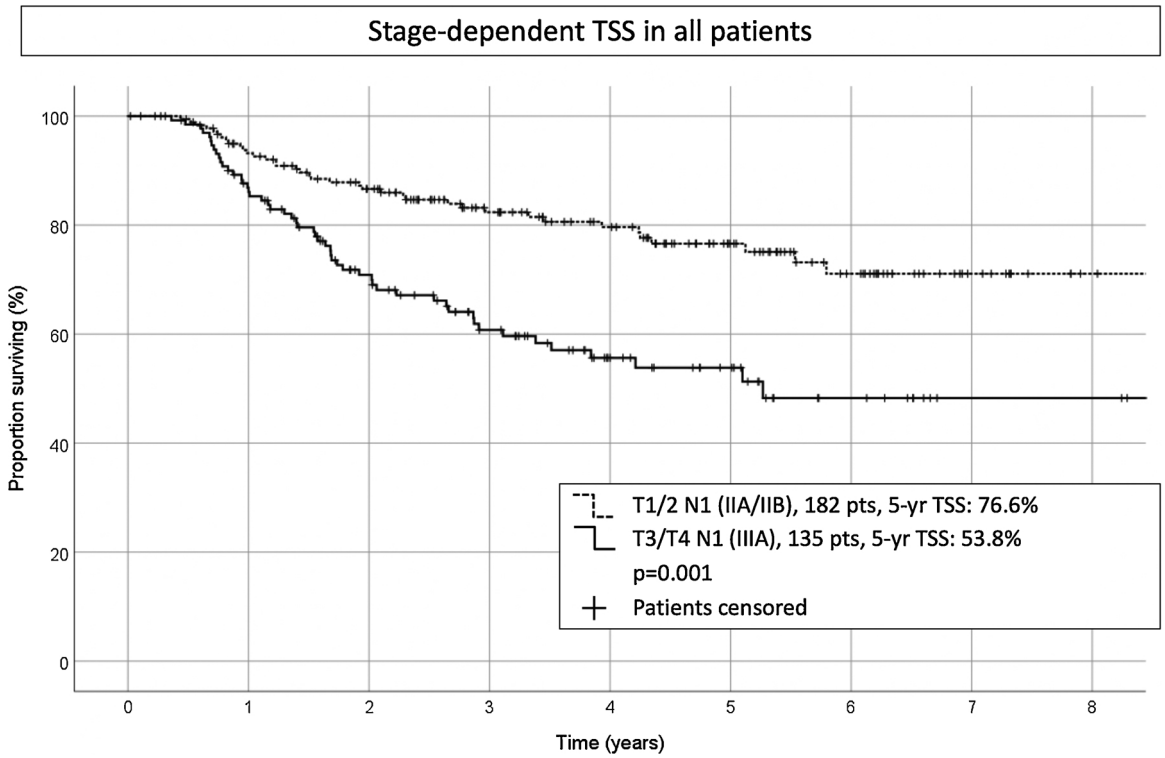

Fig. 2. Tumor stage was clearly identified as an independent factor with influence on survival irrespective of tumor histology and adjuvant therapy. Survival was significantly worse in more advanced disease (stage IIIA vs. IIA/B, p = 0.001). TSS: Tumor specific survival.

metastatic patterns, it is important to note that LN staging varies between Asia and western/European countries. Discrepancies among the "Naruke" map (Asia) [20] and the "Mountain-Dresler" modification (rest of the world) [13] harden consistent allocation to N1- and N2-level compartments (e.g., inferior border of the main stem bronchus: Naruke: N1, Mountain-Dresler: N2). These variations may explain the striking deviation of 5-year survival rates in European (36%) and Asian patients (61%) found in the IASLC database. Moreover, European information is under-represented, with only 544 pN1 patients from Norway and Serbia analyzed. In contrast, pN1 data from 2496 Asian patients, representing

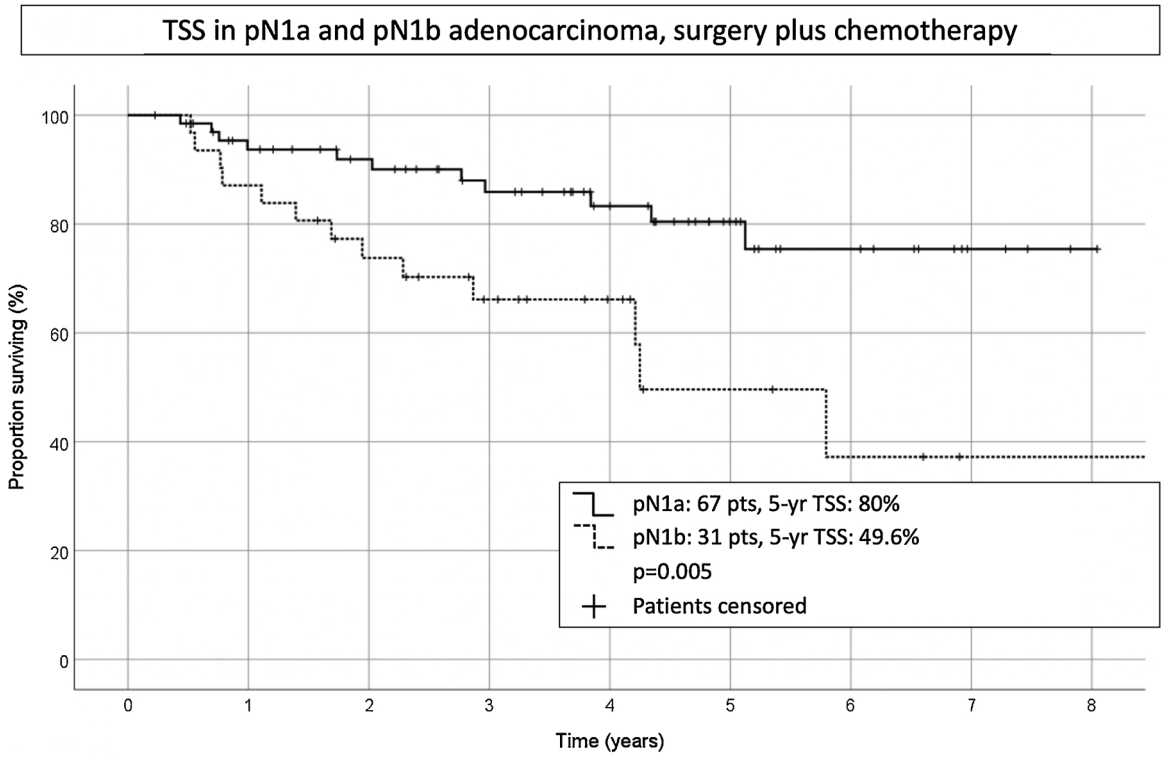

Fig. 3. Dividing pN1-patients into pN1a and pN1b was found to have prognostic impact in one subgroup of our patients: significant difference in survival could be demonstrated in patients with adenocarcinoma histology and complete adjuvant chemotherapy. TSS: Tumor specific survival.

65% of all worldwide pN1-patients, were found to be eligible for evaluation [17].

We believe that our analysis can serve as an important addition to the existing data. All pN1-patients in our series were classified according to the M—D system, in which the observed TSS of the entire group was 67.1% at 5 years. Interestingly, five-year survival in patients without adjuvant therapy was still calculated to be 57%, which is > 20% higher than the reported survival rate of the European IASLC cohort, reaching 36%. The observed discrepancy in the survival rates is difficult to explain. The large data set and its retrospective characteristics may result in incomprehensible information regarding the adjuvant therapy regimen. Furthermore, frequency and thoroughness of follow-up-intervals affect the latency to detection of recurrent disease and, therefore, influence survival. Third, individual differences regarding the invasiveness of intraoperative mediastinal nodal staging (from nodal sampling to complete compartment dissection) are prognostically relevant. A more restrictive nodal examination during surgery may underestimate true nodal extent, thus influencing subsequent therapy and course of the disease [11,21–23].

To overcome prognostic variations caused by different classification systems or discrepancies in intraoperative nodal allocations, Rusch et al. proposed to group LNs into “zones,” as described in a validated international IASLC map [14]. Following this model, we also found that N1 metastases in more than one zone (i.e., hilar and peripheral) were associated with poorer prognosis compared with single-zone involvement. However, in our series, these findings were identified as a significant prognostic factor only in adenocarcinoma, not in squamous cell carcinoma. A correlation between distribution patterns of N1 LNs and survival has been repeatedly demonstrated [24,25]; nevertheless, differences between distinct histological subtypes were not observed. In large surgical NSCLC populations, controversial results with regard to prognostic differences favoring either adenocarcinoma or squamous cell carcinoma have been reported [26–29]. Notably, these series and, as mentioned above, the IASLC database, consist of a large Asian study population. These harbor a significantly higher incidence of lung adenocarcinoma than the western or European countries, which must be taken into account during data interpretation [27,30,31]. Our series supports recent results in Caucasian pN1 NSCLC [32–34] and may serve as a valuable contribution to the existing European source data in the IASLC database. Nevertheless, the clinical impact of these findings remains debatable as long as prospective multicenter analysis of individual patient data has not been implemented. Currently, except a partial influence of the T-descriptor, adjuvant treatment decision merely follows the pathological N-status irrespective of the individual nodal burden. Consequently, upcoming revisions of the TNM-system should strongly take into account nodal subgroups in order to prospectively evaluate possibly relevant changes in treatment or follow-up strategies. In the light of recent developments in targeted and immunotherapy in advanced lung cancer, the relevance of novel therapeutic approaches should be underlined in future prospective clinical workup of nodal subgroups with prognostic relevance.

We analyzed a large surgical single-center cohort of patients with N1-positive NSCLC, and identified adenocarcinoma histology and advanced nodal extent (pN1b, multiple N1 zones affected) as poor prognostic factors. Our results support the recent survival data from pN1 patients housed in the IASLC database. For future analysis, our carefully selected data could serve as an important addition to the sparse previous European contribution to the IASLC database. We suggest that upcoming proposals for future TNM-classifications should assess the value of a more detailed stratification of N1 patients toward histology, nodal extent, and anatomical compartment distribution.

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Declaration of Competing Interest

None.

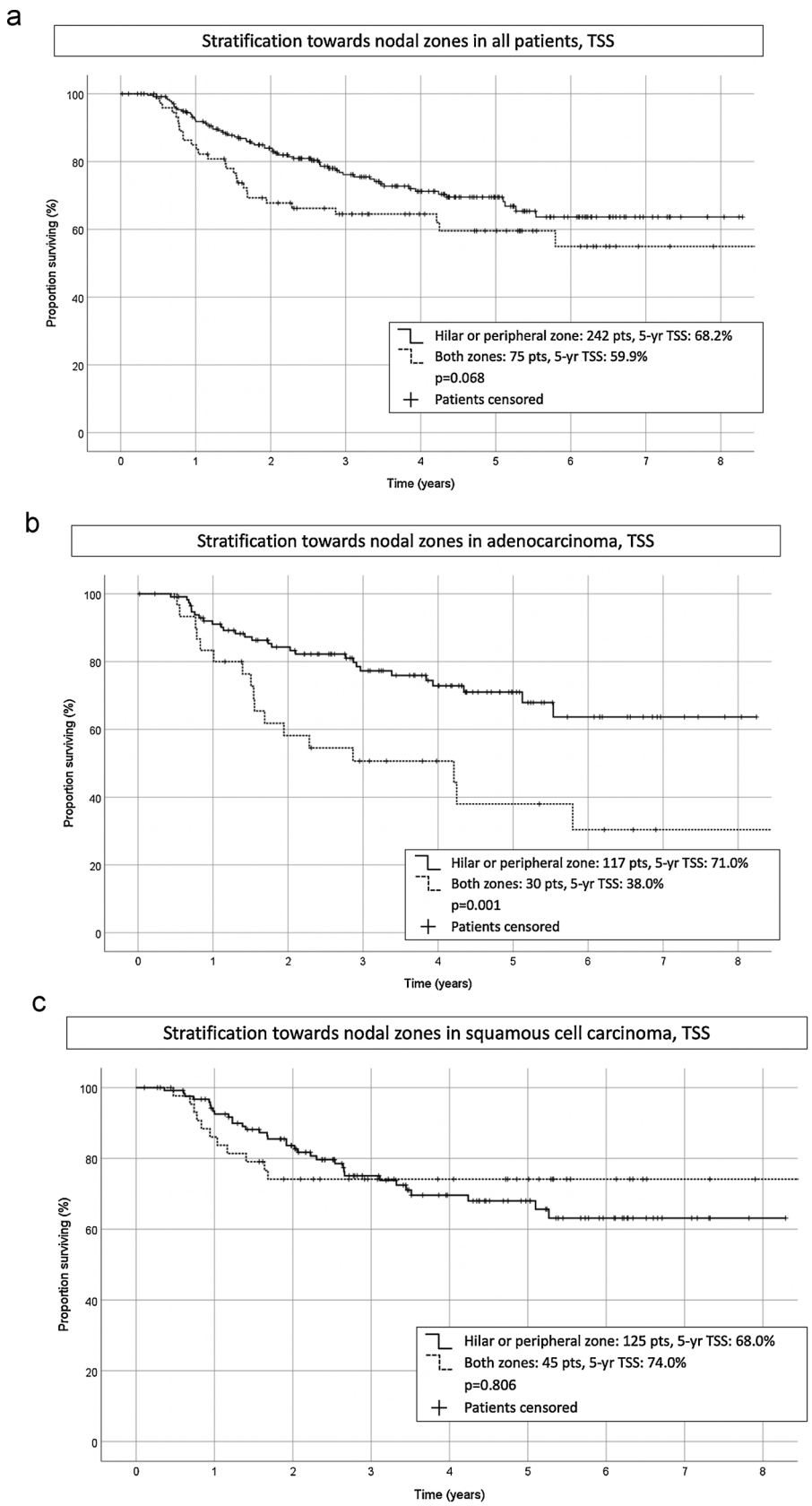

Fig. 4. Survival analysis with respect to the location based model (hilar vs. peripheral zone, according to IASLC [14]). A tendency towards poorer prognosis was found in patients with multiple nodal zones involved (A). Univariate analysis after stratification towards histological subtype showed significant poorer survival in patients with adenocarcinoma (B, p = 0.001). No difference was found in patients with squamous cell carcinoma (C, p = 0.806).

Table 2
Multivariate analysis identified ECOG-stage, tumor stage, histology, chemotherapy treatment and nodal tumor burden as individual factors with prognostic impact.

Comparative factor	Tumor specific survival (TSS)		
	HR	[95%-CI]	p-value
Age	0.996	[0.97-1.02]	0.74
ECOG			
0	1	Reference	–
1	1.904	[1.16-3.11]	0.01
Histology			
Squamous cell carcinoma	1	Reference	–
Adenocarcinoma	1.896	[1.19-3.00]	0.006
Adjuvant Chemotherapy			
No	1	Reference	–
Yes	0.516	[0.31-0.84]	0.008
Tumor Stage			
IIA/B	1	Reference	–
IIIA	2.301	[1.46-3.62]	< 0.0001
Metastatic nodal station			
pN1a (single)	0.383	[0.13-1.06]	0.066
pN1b (multiple)	1	Reference	–
Metastatic nodal region			
Single zone (hilar or peripheral)	1	Reference	–
Multiple zone (hilar and peripheral)	3.43	[1.17-9.62]	0.024
Extent of resection			
Lobectomy	1		
Bilobectomy/Pneumonectomy	1.334	[0.81-2.18]	0.25

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CONFIRMATION OF CONGRUENCY BETWEEN PRINTED AND ELECTRONIC VERSION OF THE DOCTORAL THESIS

I hereby declare that the electronic version of the submitted thesis, entitled

“Deep phenotyping of patient cohorts with thoracic malignancies”

is congruent with the printed version both in content and format.

Heidelberg, 12.01.2021	Laura Klotz
Place, date	Signature doctoral candidate