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High tumor mutation burden predicts favorable outcome among patients with aggressive histological subtypes of lung adenocarcinoma: A population-based single-institution study

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Abstract

Objectives: Tumor mutation burden (TMB) is an emerging predictive cancer biomarker. Few studies have addressed the prognostic role of TMB in non-small cell lung carcinoma, with conflicting results. Moreover, the association of TMB with different histological subtypes of lung adenocarcinoma has hitherto not been systematically evaluated. Here we studied the prognostic value of TMB and its distribution in different histological subtypes of lung adenocarcinomas in a retrospective cohort using the most recent updated classification guidelines.

Materials and methods: 176 surgically resected stage I–IV lung adenocarcinomas were histologically reclassified according to WHO 2015 guidelines. A modified classification subdividing the acinar subtype into classic acinar, complex glandular and cribriform subtypes was further applied and potentially prognostic histopathological characteristics such as tumor-infiltrating lymphocytes were evaluated. 148 patients with stage I–III tumors and complete follow-up data were included in the survival analyses. TMB was determined by a commercial next generation sequencing panel from 131 tumors, out of which 105 had survival data available.

Results: Predominant micropapillary, solid and complex glandular as well as nonpredominant cribriform histological subtypes were associated with significantly shorter survival. High TMB concentrated in micropapillary, solid and acinar predominant subtypes. Interestingly, TMB $\geq$ 14 mutations/MB conferred a stage- and histology-independent survival benefit compared to TMB < 14 in multivariable analysis for overall (HR 0.284, 95% CI 0.14–0.59, P=0.001) and disease-specific survival (HR 0.213, 95% CI 0.08–0.56, P=0.002).

Conclusion: TMB was an independent biomarker of favorable prognosis in our cohort of lung adenocarcinoma despite being associated with predominant histological subtypes considered aggressive.

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Keywords: Non-small cell lung cancer, Lung adenocarcinoma, Tumor mutation burden, Histological subtype, Prognostic biomarker

Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide [1]. The prognosis remains poor, with a 5-year survival of 10–20% in most countries [2]. Almost half of all lung cancers represent adenocarcinomas [3]. The histological composition of these

Abbreviations: LVI, lymphovascular invasion, STAS, spread through air spaces, TMB, nonsynonymous tumor mutation burden defined as mutations per megabase of coding DNA, VPI, visceral pleural invasion

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morphologically heterogeneous tumors is an established prognostic factor together with stage [4].

The International Association for the Study of Lung Cancer/The American Thoracic Society/The European Respiratory Society (IASLC/ATS/ERS) classification adopted by World Health Organization (WHO) in 2015 [4] recommends classifying adenocarcinomas by designating them a predominant as well as one or more nonpredominant histological subtypes. Lepidic predominant adenocarcinomas are associated with favorable survival, acinar and papillary predominant adenocarcinomas with intermediate prognosis, and solid and micropapillary predominant subtypes with poor prognosis [4]. In addition, there is some evidence that acinar morphology with complex glandular growth patterns is associated with a poorer prognosis than classic acinar pattern suggesting that separating these morphologies may provide additional prognostic and/or predictive information [5–10].

Nonsynonymous tumor mutation burden (TMB), defined as mutations per megabase of coding DNA, is a promising biomarker in various cancers. Lung adenocarcinomas are genetically diverse with a generally high number of somatic mutations [11–13]. In spite of this, so far only few studies have addressed the prognostic role of TMB in lung cancer, with contradictory outcomes. One study reported improved prognosis in patients with non-small cell carcinomas (NSCLC) harboring high TMB [14], whereas two studies defined high TMB as a poor prognostic factor in NSCLC [15] or lung adenocarcinoma [16]. Because of the scant data and conflicting results, no generally approved cutoff to stratify patients into high and low burden groups has yet been established.

In the current study, we aimed to correlate the association of TMB with histological subtypes and patient survival in a systematically collected retrospective single institution cohort of lung adenocarcinomas. In parallel, the prognostic role of complex glandular structures of acinar adenocarcinoma and tumor-infiltrating lymphocytes were tested. Our results suggest that high TMB, although enriched in histological subtypes considered aggressive, is an independent predictor of favorable survival after surgery.

Materials and methods

Patients

Our retrospective cohort consisted of 176 surgically resected stage I–IV invasive lung adenocarcinomas from patients operated in Turku University Hospital between 2003 and 2017. Two patients had received neoadjuvant chemotherapy while none had received preoperative radiotherapy or any immunotherapy during the follow-up period. 43 patients received adjuvant chemotherapy and 8 adjuvant radiotherapy. We collected clinical and histopathological data from the electronic patient registries, and one experienced pulmonologist (HV) restaged the tumors according to the current 8th edition of TNM classification [4] (Table 1). The day of death and causes of death were acquired through Statistics Finland (Helsinki, Finland), with data available until the end of 2016. Smoking status was assigned based on the patient registry entries. We excluded patients from survival analyses based on one or more of the following criteria: operation in 2017, incomplete clinical follow-up data, death within 30 postoperative days, and macroscopic (R2) residual disease (Fig. 1). The collection of clinical patient data was approved by the administration of Hospital District of Southwest Finland (T150/16) and the use of tissue material was approved by the Scientific Steering Committee of Auria Biobank (AB14-8689). The study was conducted in collaboration with Auria Biobank and Roche (Espoo, Finland).

Histopathological evaluation

We re-evaluated all histological material from resected lung cancers operated in our institute in 2003–2017 and selected adenocarcinomas

Table 1. Clinical characteristics of the cohort and their effects on 5-year overall survival and disease specific survival. Univariable Cox regression and Kaplan-Meier (KM) analyses. OS = overall survival; DSS = disease specific survival. Bold values indicate P < 0.05.

Univariable analysis				Univariable OS (Cox)			Univariable DSS (Cox)			5-year DSS (%) (KM)		5-year DSS (%) (KM)	
		No. of patients (%) (n = 176)	No. of patients in survival analyses (%) (n = 148)	HR	95% CI	P-value	HR	95% CI	P-value		P-value		P-value
Mean age at operation		66.4											
Sex	Female	81 (46.0)	69 (46.6)	REF			REF			56.6	**0.041**	65.0	0.195
	Male	95 (54.0)	79 (53.4)	1.613	1.02–2.56	**0.043**	1.428	0.83–2.45	0.197	46.2		57.5	
Smoking	No	38 (21.6)	34 (23.0)	REF			REF			46.9	0.816	60.3	0.986
	Yes	137 (77.8)	114 (77.0)	0.937	0.54–1.63	0.816	0.994	0.51–1.93	0.986	52.1		61.3	
	Unknown	1 (0.6)											
Type of surgery	Sublobar resection	12 (6.8)	9 (6.1)	REF			REF			38.1	0.380	59.3	0.712
	Lobectomy	114 (64.8)	96 (64.9)	0.762	0.30–1.91	0.563	0.930	0.29–3.02	0.904	47.4		56.9	
	Bilobectomy	44 (25.0)	40 (27.0)	0.505	0.19–1.36	0.177	0.655	0.19–2.29	0.507	63.5		71.4	
	Pneumonectomy	5 (2.8)	3 (2.0)	0.815	0.158–4.21	0.807	0.699	0.07–6.74	0.757	33.3		50.0	
	Unknown	1 (0.6)											
Stage (TNM8)	I	95 (54.0)	87 (58.8)	REF			REF			64.7	**0.002**	77.1	**<0.001**
	II	38 (21.6)	32 (21.6)	2.174	1.28–3.70	**0.004**	3.507	1.88–6.54	**<0.001**	35.2		39.4	
	III	37 (21.0)	29 (19.6)	2.317	1.31–4.10	**0.004**	3.465	1.76–6.82	**<0.001**	29.2		39.4	
	IV	5 (2.8)	Excluded										
	Unknown	1 (0.6)											
Residual disease	No	147 (83.5)	136 (91.9)	REF			REF			54.3	**0.001**	64.4	**0.001**
	Microscopic	12 (6.8)	12 (8.1)	3.084	1.51–6.30	**0.002**	3.501	1.62–7.55	**0.001**	18.2		22.7	
	Macroscopic	15 (8.5)	Excluded										
	Unknown	2 (2.3)	0										

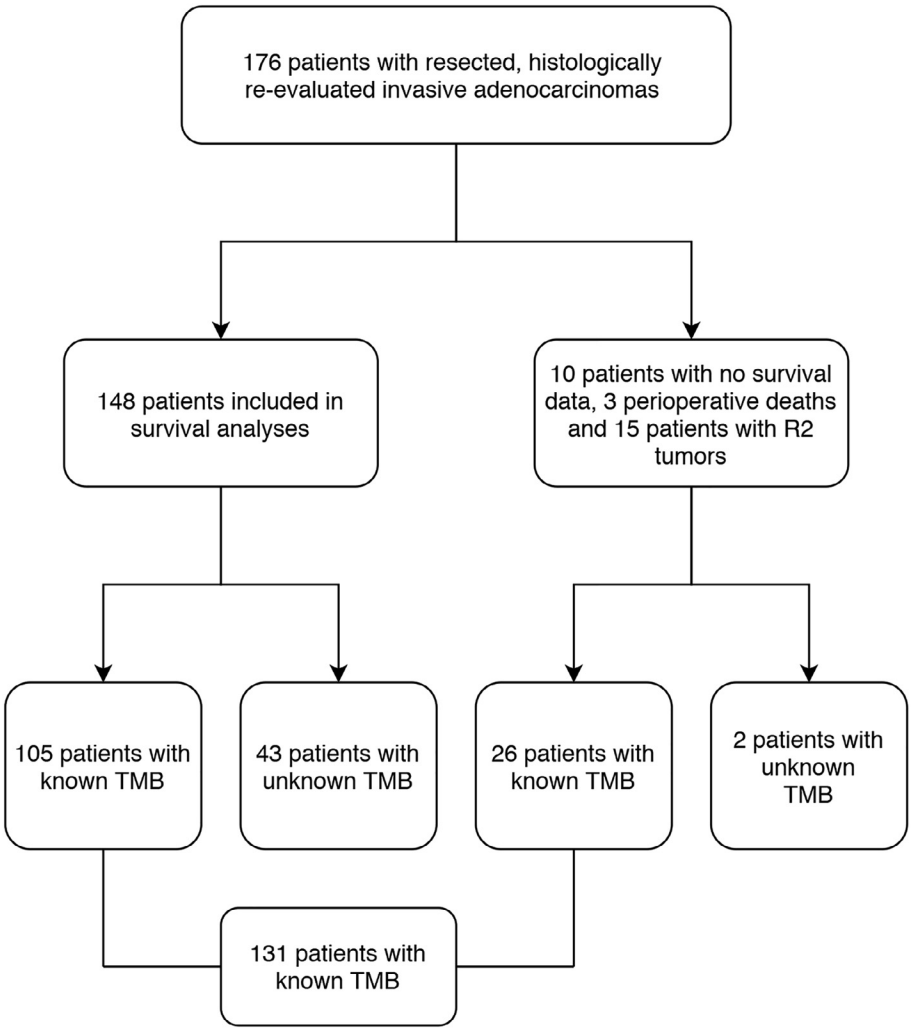

Fig. 1. Flow chart of cases included in different parts of the study. TMB = tumor mutation burden; R2 tumors = patients with macroscopic residual disease at surgery.

based on histopathological morphology and/or appropriate immunohistochemical and/or Periodic acid-Schiff (PAS) staining according to current WHO guidelines. All available slides were digitally scanned (Pannoramic 250 Flash, 3DHistech, Budapest, Hungary), uploaded onto the University of Turku digital microscopy web portal (casecenter.utu.fi) and viewed with Case Viewer software (3DHistech). As per our institution's practice, the majority of the slides were originally stained with van Gieson and the rest with hematoxylin and eosin (HE). Two pathologists (ET and PT) independently performed the histopathological evaluation, blinded to clinical data. In case of a discrepancy, inspectors reached a consensus after reviewing the case together. The median number of tumor slides per case was 3 (range 1–12). The presence of visceral pleural invasion (VPI), lymphovascular invasion (LVI), spread through air spaces (STAS), and any tumor necrosis were visually determined. STAS status was classified as either no STAS or any STAS.

We evaluated the abundance of tumor-infiltrating lymphocytes (TILs) in both stromal and epithelial components of the invasive front and the center of the tumor. Unless otherwise available, one representative HE-stained tumor slide per case was prepared for evaluation. Researchers experienced in counting TILs (AA and HM) estimated the percentage of surface area occupied by TILs according to the guidelines by the International Immuno-Oncology Biomarkers Working Group [17].

Tumor mutation burden

FoundationOne (Foundation Medicine, Inc., Cambridge, MA, USA) comprehensive genomic profiling was performed on all the formalin-fixed paraffin-embedded samples that met the analysis requirements. Ten 5 μm thick paraffin sections on charged and unbaked slides were used for the analysis. Cases with an insufficient number of non-necrotic tumor cells and those limited to a single tumor block per patient were omitted. The analysis method was conducted as previously described and validated by Frampton et al. [18]. Similarly, TMB was analyzed as previously described [19].

Statistical analyses

The clinical and histopathological data were correlated with the χ^2 test and Fischer's exact test. Interobserver variability was evaluated with Cohen's Kappa statistic. Overall survival (OS) and disease-specific (DSS) survival were estimated using Kaplan-Meier analysis. The univariable effects of clinicopathological parameters and TMB on survival were assessed with the log-rank test and multivariable survival analysis with the Cox proportional hazards regression model. P-values less than 0.05 were considered statistically significant. Interaction terms in the Cox

model were evaluated. Statistical analyses were performed with SPSS (IBM, version 25, 2017) and JMP13 (SAS Institute Inc, version 13.1.0, 2016).

Results

Patient characteristics

Out of 176 patients, 95 (54.0%) were male and 81 (46.0%) were female. In total 137 (77.8 %) patients had a history of smoking (88.3% of men, 66.7% of women). The mean age at the time of operation was 66.4 years, and the most common type of operation was lobectomy (114, 64.8%), followed by bilobectomy (44, 25.0%), sublobar resection (12, 6.8%) and pneumonectomy (5, 2.8%). 147 patients (83.5%) underwent complete (R0) resection (Table 1).

Histological subtyping

Among the 176 adenocarcinomas re-evaluated, the most common predominant histological subtype was acinar (48.9%), followed by solid (24.4%), lepidic (6.8%), invasive mucinous (5.7%), papillary (5.1%), micropapillary (4.0%) and colloid (1.7%) (Fig. 2 and Table 2). Additionally, there were 4 cases of mixed mucinous/non-mucinous adenocarcinoma with a predominant mucinous component (2.3%) and one case of fetal (0.6%) and enteric (0.6%) adenocarcinoma each. When acinar predominant carcinoma was further subdivided into classic acinar, complex glandular and cribriform patterns, complex glandular was the most prevalent subtype comprising 26.7% of all tumors and 54.7% of acinar adenocarcinomas, followed by classic acinar (17.0% and 34.9%, respectively) and cribriform (5.1% and 10.5%, respectively). (Table 2) In general, most adenocarcinomas comprised multiple histological subtypes: 49 cases (27.8%) had 2, 58 cases (33.0%) had 3, 27 cases (15.3%) had 4, and 6 cases (3.4%) had 5 subtypes. Only 36 tumors (20.5%) showed one pure subtype. Interobserver variability for predominant subtypes expressed by Cohen's κ coefficient was 0.650 ($P < 0.001$), consistent with good interobserver variability. Histological subtypes were not associated with VPI, LVI or STAS ($P > 0.05$).

Tumor mutation burden

TMB was successfully determined from 131 adenocarcinomas. The median somatic mutation rate was 7.02/MB, similar to previous studies [11,20]. Several cutoff values were evaluated, as described in the survival analyses section. Using 14 mutations/MB as a cutoff, mutation burden ≥14/MB (high TMB) was detected in 31 (23.7%) cases: 2/4 (50%) micropapillary, 9/29 (31.0%) solid, 19/67 (28.4%) acinar and 1/10 (10.0%) lepidic predominant adenocarcinomas. When acinar adenocarcinomas were further subdivided, high TMB was present in 4/8 (50%) cribriform, 13/36 (36.1%) complex glandular, and 2/23 (8.7%) classic acinar predominant tumors. None of the tumors with mucinous or papillary predominant subtype histology had high TMB (Table 2). There was no association between TMB status and stage, VPI, LVI or STAS ($P > 0.05$).

Tumor-infiltrating lymphocytes

Since high TMB may increase the abundance of neoantigens and immunological response within the tumor, the number of TILs was quantified. We were able to determine the invasive front and the percentage of TILs in total from 171 tumors (97.2%). Of these, 126 patients (73.7%) had a low number of TILs (≤20% of the surface area), and 45 (26.3%) had a high number of TILs (>20%) within the invasive front stroma. There was no statistically significant association between the TIL groups and predominant histological subgroups (P = 0.567) or TMB status (P = 0.287) (Table 3). Similarly, TIL groups were not associated with stage, VPI, LVI or STAS ($P > 0.05$).

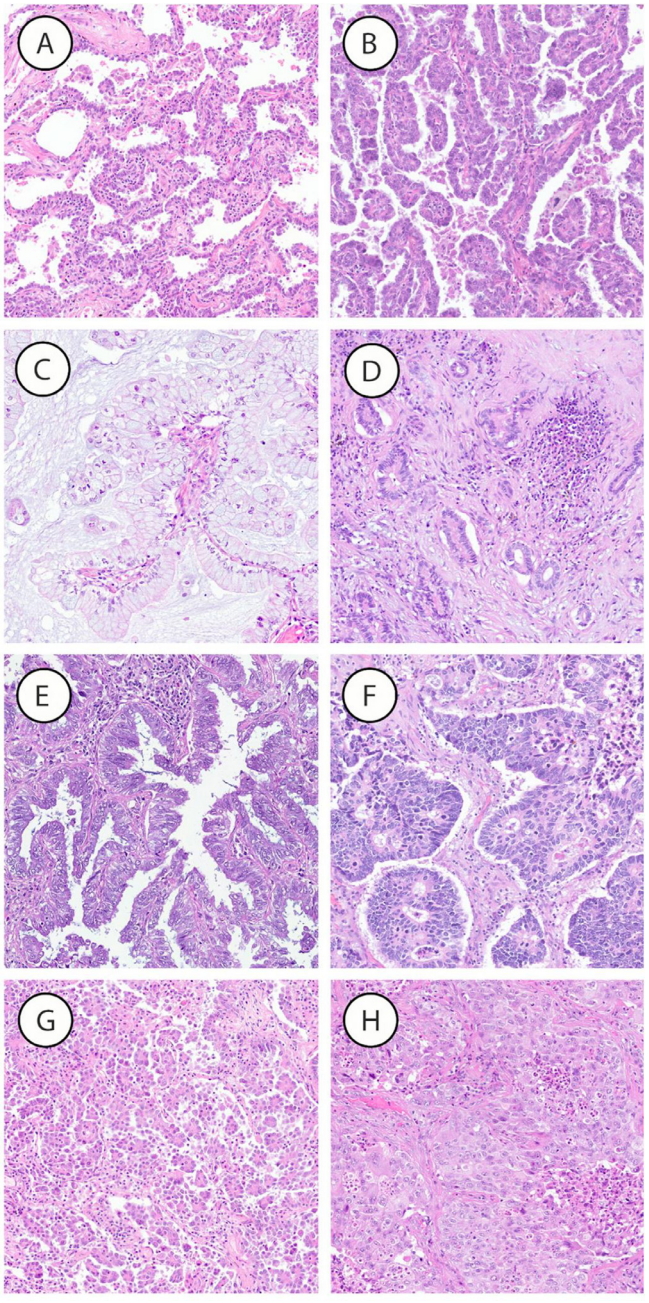

Fig. 2. Representative examples of different histological subtypes in lung adenocarcinoma. A. lepidic, B. papillary, C. mucinous, D. classic acinar, E. complex glandular, F. cribriform, G. micropapillary, H. solid (x20 magnification).

Survival analyses

In total, 148 out of 176 patients were included in the survival analyses after applying the exclusion criteria (Fig. 1 and Tables 1–3). The median clinical follow-up was 45 months (0.6–167.6 months). The 5-year OS rate was 51.0%, and the 5-year DSS rate 61.2%. Fifty-five patients (37.2%) died of lung adenocarcinoma and 22 patients (14.9%) of other causes

Table 2. Predominant histological subtypes and their effect on survival. Univariable Kaplan-Meier (KM) analysis. IASLC/ATS/ERS = The International Association for the Study of Lung Cancer/The American Thoracic Society/The European Respiratory Society; OS = overall survival; DSS = disease specific survival, TMB = tumor mutation burden. Bold values indicate P < 0.05.

Predominant pattern (IASLC/ATS/ERS)	Predominant pattern (modified)	No. of patients (%) (n = 176)	No. of patients with survival data (%) (n = 148)	No. of patients with high TMB/all patients with TMB data (%) (n = 31/131)	No. of patients with survival and TMB data (%) (n = 105)	5-year OS (%) (KM)	5-year DSS (%) (KM)
P-value (IASLC/ATS/ERS)						**P = 0.028**	**P = 0.001**
P-value (modified)						**P = 0.047**	**P = 0.002**
Lepidic		12 (6.8)	9 (6.1)	1/10 (10)	7 (6.7)	72.9	83.3
Acinar		86 (48.9)	73 (49.3)	19/67 (28.4)	54 (51.4)	55.6	67.9
	Classic acinar	30 (17.0)	26 (17.6)	2/23 (8.7)	19 (18.1)	58.1	72.9
	Complex glandular	47 (26.7)	39 (26.4)	13/36 (36.1)	28 (26.7)	47.5	57.2
	Cribriform	9 (5.1)	8 (5.4)	4/8 (50)	7 (6.7)	85.7	100
Papillary		9 (5.1)	9 (6.1)	0/9 (0)	9 (8.6)	66.7	66.7
Micropapillary		7 (4.0)	6 (4.1)	2/4 (50)	3 (2.9)	44.4	44.4
Solid		43 (24.4)	35 (23.6)	9/29 (31.0)	22 (21.0)	36.7	43.5
Enteric		1 (0.6)	1 (0.7)	N/A	N/A	100	100
Fetal		1 (0.6)	1 (0.7)	0/1 (0)	1 (1.0)	0	0
Mucinous histology		17 (9.7)	14 (9.5)	0/11 (0)	9 (8.6)	50.3	68.6
	Invasive mucinous	10 (5.7)	8 (5.5)	0/6 (0)	5 (4.8)	37.5	62.5
	Mixed mucinous, mucinous predominant	4 (2.3)	3 (2.0)	0/4 (0)	3 (2.9)	0	0
	Colloid	3 (1.7)	3 (2.0)	0/1 (0)	1 (1.0)	100	100

during the follow-up period. As expected, clinical stage strongly predicted both OS (HR 2.174, 95% CI 1.28–3.70 for stage II and HR 2.317, 95% CI 1.31–4.10 for stage III, P = 0.004) and DSS (HR 3.507, 95% CI 1.88–6.54 for stage II and HR 3.465, 95% CI 1.76–6.82 for stage III, $P < 0.001$) (Table 1). Similarly, VPI predicted a poor OS (HR 1.715, 95% CI 1.07–2.76, P = 0.026) and DSS (HR 1.943, 95% CI 1.12–3.38, P = 0.018) when compared to patients without VPI. The presence of tumor necrosis was associated with a shorter DSS (HR 1.713, 95% CI 1.00–2.92, P = 0.048) while LVI or STAS did not affect survival. (Table 3)

Survival analyses for predominant histological subtyping

Predominant histological subtypes divided the cohort into two distinct survival groups. Following the IASLC/ATS/ERS classification, lepidic, acinar, papillary and enteric predominant adenocarcinomas, and adenocarcinomas with mucinous histology represented a group of favorable prognosis. On the other hand, micropapillary, solid and fetal predominant subtypes were indicators of poor OS (HR 2.221, 95% CI 1.40–3.53, P = 0.001) and DSS (HR 3.062, 95% CI 1.80–5.22, $P < 0.001$) (Fig. 3A and Table 3). The survival groups correlated with stage as there were more early stage tumors in the favorable prognosis group (80.5%, 65.6% and 51.7% of stage I, II and III tumors with favorable histology, respectively, P = 0.008). Despite this, the survival groups were stage- and TMB-independent predictors of both OS (HR 3.946, 95% CI 1.97–7.92, $P < 0.001$) and DSS (HR 4.875, 95% CI 2.13–11.15, $P < 0.001$) in multivariable analysis (Table 3).

Survival analyses for modified histological subtyping

Dividing the acinar predominant subtype into classic acinar, complex glandular, and cribriform subtypes formed three distinct survival groups in univariable analysis for DSS but not for OS. The group with the most favorable survival consisted of lepidic, classic acinar, cribriform, and enteric predominant subtypes. Complex glandular, papillary, and mucinous histology subtypes formed a group with an intermediate DSS (HR 2.433, 95% CI 1.04–5.67, P = 0.04) while solid, micropapillary, and fetal predominant subtypes were indicators of poor DSS (HR 5.587, 95% CI 2.41–12.95, $P < 0.001$) (Fig. 3B and Table 3). Although significant in univariable analysis, the modified subtyping did not reach independent statistical significance in multivariable analysis.

Of note, the patients with tumors featuring a nonpredominant cribriform subtype had a markedly worse OS (HR 2.588, 95% CI 1.50–4.46, P = 0.001) and DSS (HR 3.032, 95% CI 1.64–5.60, $P < 0.001$) than tumors without nonpredominant cribriform component (Fig. 3C and Table 3) whereas other nonpredominant subtypes had no statistically significant effect on survival (data not shown).

Survival analyses for tumor mutation burden

After applying the exclusion criteria, 105 cases with TMB data were included in the survival analysis (Fig. 1 and Table 3). We evaluated the impact of several cutoff values of TMB (7–20 mutations/MB) on survival and found the most significant difference when using 14 mutations/MB as a cutoff. High TMB (≥14 mutations/MB) was a favorable prognostic factor in univariable analysis for both OS (HR 0.435, 95% CI 0.22–0.85, P = 0.015) and DSS (HR 0.351, 95% CI 0.15–0.83, P = 0.021) (Fig. 3D and Table 3). Moreover, TMB retained its prognostic value in multivariable analysis for OS (HR 0.284, 95% CI 0.14–0.59, P = 0.001) and DSS (HR 0.213, 95% CI 0.08–0.56, P = 0.002) independent of stage and histological subtype (Table 3). Importantly, the frequencies of adjuvant therapies did not differ between the low and high TMB

Table 3. Histopathological characteristics of the cohort and their effects on 5-year overall survival and disease specific survival. Univariable and multivariable Cox regression and Kaplan-Meier (KM) analyses. OS = overall survival; DSS = disease specific survival; IASLC/ATS/ERS = The International Association for the Study of Lung Cancer/The American Thoracic Society/The European Respiratory Society; TMB = tumor mutation burden; TILs = tumor infiltrating lymphocytes; VPI = visceral pleural invasion; LVI = lymphovascular invasion; STAS = spread through air spaces. Bold values indicate P < 0.05.

Univariable analysis				Univariable OS (Cox)			Univariable DSS (Cox)			5-year OS (%) (KM)		5-year DSS (%) (KM)	
		No. of patients (%) (n = 176)	No. of patients in survival analyses (%) (n = 148)	HR	95% CI	P-value	HR	95% CI	P-value		P-value		P-value
Predominant pattern (IASLC/ATS/ERS)	Favorable prognosis	125 (71.0)	106 (71.6)	REF			REF			56.7	**0.001**	68.5	**<0.001**
	Poor prognosis	51 (29.0)	42 (28.4)	2.221	1.40–3.53	**0.001**	3.062	1.80–5.22	**<0.001**	36.8		42.4	
Predominant pattern (modified)	Favorable prognosis	52 (29.5)	44 (29.7)	REF			REF			65.4	**0.002**	79.9	**<0.001**
	Intermediate prognosis	73 (41.5)	62 (41.9)	1.385	0.77–2.51	0.281	2.433	1.04–5.67	**0.04**	50.6		60.7	
	Poor prognosis	51 (29.0)	42 (28.4)	2.708	1.49–4.94	**0.001**	5.587	2.41–12.95	**<0.001**	36.8		42.4	
Nonpredominant cribriform subtype	No	150 (85.2)	126 (85.1)	REF			REF			56.4	**<0.001**	66.5	**<0.01**
	Yes	26 (14.8)	22 (14.9)	2.588	1.50–4.46	**0.001**	3.032	1.64–5.60	**<0.001**	21.5		29.9	
TMB	Low	100 (56.8)	79 (53.4)	REF			REF			41.3	**0.013**	55.2	**0.016**
	High	31 (17.6)	26 (17.6)	0.435	0.22–0.85	**0.015**	0.351	0.15–0.85	**0.021**	72.8		80.2	
	Unevaluable	45 (25.6)	43 (29.1)										
Combined histological and TMB groups	Group 1	18 (10.2)	18 (12.2)	REF			REF			78.8	**<0.001**	84.4	**<0.001**
	Group 2	69 (39.2)	69 (46.6)	2.470	1.03–5.91	**0.042**	2.972	0.88–10.01	**0.079**	49.7		64.4	
	Group 3	18 (10.2)	18 (12.2)	13.374	4.67–38.27	**<0.001**	21.533	5.57–83.23	**<0.001**	0		0	
	Unevaluable	71 (40.3)	43 (29.1)										
TILs (invasive front)	≤20 %	126 (71.6)	106 (71.6)	REF			REF			44.6	**0.019**	55.0	**0.031**
	>20 %	45 (25.6)	38 (25.7)	0.497	0.27–0.90	**0.022**	0.464	0.22–0.95	**0.035**	70.5		76.1	
	Unevaluable	5 (2.3)	4 (2.7)										
VPI	No	117 (66.5)	99 (66.9)	REF			REF			56.2	**0.024**	66.3	**0.016**
	Yes	52 (29.5)	43 (29.1)	1.715	1.07–2.76	**0.026**	1.943	1.12–3.38	**0.018**	35.1		46.5	
	Unevaluable	7 (4.0)	6 (4.1)										
LVI	No	98 (55.7)	86 (58.1)	REF			REF			56.4	0.080	63.2	0.254
	Yes	78 (44.3)	62 (41.9)	1.491	0.95–2.34	0.082	1.364	0.80–2.33	0.256	43.2		58.3	
STAS	No	65 (36.9)	51 (34.5)	REF			REF			52.9	0.305	63.9	0.368
	Yes	105 (59.7)	91 (61.5)	1.298	0.79–2.14	0.306	1.315	0.72–2.39	0.369	49.8		60.4	
	Unevaluable	6 (3.4)	6 (4.1)										
Necrosis	No	91 (51.7)	78 (52.7)	REF			REF			56.9	0.125	66.4	**0.046**
	Yes	85 (48.3)	70 (47.3)	1.418	0.91–2.22	0.127	1.713	1.00–2.92	**0.048**	44.5		55.1	
Multivariable analysis				Multivariable OS (Cox)	Multivariable DSS (Cox)								
				HR	95% CI	P-value	HR	95% CI	P-value				
Stage (TNM8)	I		87 (58.8)	REF			REF						
	II		32 (21.6)	2.355	1.18–4.72	0.016	4.357	1.82–10.46	**0.001**				
	III		29 (19.6)	1.773	0.88–3.57	0.108	3.120	1.29–7.58	**0.012**				
	IV		Excluded										
Predominant pattern (IASLC/ATS/ERS)	Favorable prognosis		106 (71.6)	REF			REF						

Table 3 (*continued*)

Univariable analysis				Univariable OS (Cox)			Univariable DSS (Cox)			5-year OS (%) (KM)		5-year DSS (%) (KM)	
		No. of patients (%) (n = 176)	No. of patients in survival analyses (%) (n = 148)	HR	95% CI	P-value	HR	95% CI	P-value		P-value		P-value
	Poor prognosis		42 (28.4)	3.946	1.97–7.92	**<0.001**	4.875	2.13–11.15	**<0.001**				
TMB	Low		79 (53.4)	REF			REF						
	High		26 (17.6)	0.284	0.14–0.59	**0.001**	0.213	0.08–0.56	**0.002**				
	Unevaluable		43 (29.1)										

groups in the whole cohort (36.4% vs 33.3% for chemotherapy and 5.7% vs 10.0% for radiotherapy, $P > 0.05$; Supplementary Table 1) or among patients with relapsed disease (41.2% vs 31.3% for chemotherapy and 7.8% vs 12.5% for radiotherapy, $P > 0.05$; Supplementary Table 2). When using either 10 or 20 mutations/MB as a cutoff, there was a trend for improved prognosis among patients with high TMB but these differences did not reach statistical significance for either OS (HR 0.751, 95% CI 0.429–1.314, P = 0.316 for cutoff of 10 mutations/MB and HR 0.527, 95% CI 0.247–1.123, P = 0.097 for cutoff of 20 mutations/MB) or DSS (HR 0.686, 95% CI 0.343–1.370, P = 0.285 for cutoff of 10 mutations/MB and HR 0.502, 95% CI 0.194–1.299, P = 0.155 for cutoff of 20 mutations/MB).

Combining the data of the TMB groups with histological subtyping according to the IASLC/ATS/ERS classification further separated the patients into three distinct subgroups: (1) the tumors with a favorable histological subtype and high TMB, (2) the tumors with a favorable histological subtype and low TMB combined with the tumors with a poor histological subtype and high TMB, and (3) the tumors with a poor histological subtype and low TMB. Compared to the group 1 with an excellent prognosis, OS was significantly reduced in group 2 (HR 2.470, 95% CI 1.03–5.91, P = 0.042) and particularly poor in group 3 (HR 13.374, 95% CI 4.67–38.27, $P < 0.001$), although with wide confidence intervals. Similarly, there was a clear statistically significant difference in DSS between groups 1 and 3 (HR 21.533, 95% CI 5.57–83.23, $P < 0.001$), while the difference between groups 1 and 2 slightly failed to reach statistical significance (HR 2.972, 95% CI 0.88–10.01, P = 0.079). (Fig. 3E and Table 3)

Survival analyses for TILs

Of all the compartments evaluated for TILs, only stromal TILs at the invasive front of the tumor showed any prognostic significance in univariable analysis. After evaluating several cutoff points (5%, 10%, 15%, 20%, 25% and 30%), the best prognostic value was reached with a cutoff of 20%. A high TIL count at the invasive front stroma (>20% of the surface area) was associated with an improved OS (HR 0.497, 95% CI 0.27–0.90, P = 0.022) and DSS (0.464, 95% CI 0.22–0.95, P = 0.035) when compared to tumors with a low TIL count (Fig. 3F and Table 3). This association, however, did not reach statistical significance in multivariable analysis. Moreover, the abundance of TILs did not correlate with TMB (P = 0.287).

Discussion

In this study, we evaluated the prognostic value of TMB on survival of lung adenocarcinoma patients after surgery with curative intent. Furthermore, we studied the distribution of TMB in different histological subtypes in lung adenocarcinoma, an association we believe has not previously been reported in the literature. In addition to an established histological subtyping system by IASLC/ATS/ERS [4], we used a modified subtyping scheme to identify prognostically distinct subsets of acinar predominant adenocarcinoma. In our cohort, high TMB was associated with significantly improved survival. Acinar, micropapillary, and solid predominant subtypes were more prone to have high TMB, while tumors with predominant lepidic, papillary, and mucinous histology had few or no cases with high TMB. Additionally, classic acinar tumors had high TMB less frequently than tumors with complex glandular or cribriform predominant histology. In conclusion, high TMB was enriched in predominant subtypes considered aggressive.

The current IASLC/ATS/ERS classification defines the acinar growth pattern as ``round to oval-shaped glands with a central luminal space surrounded by tumor cells'' [4]. In our cohort, however, a large proportion of

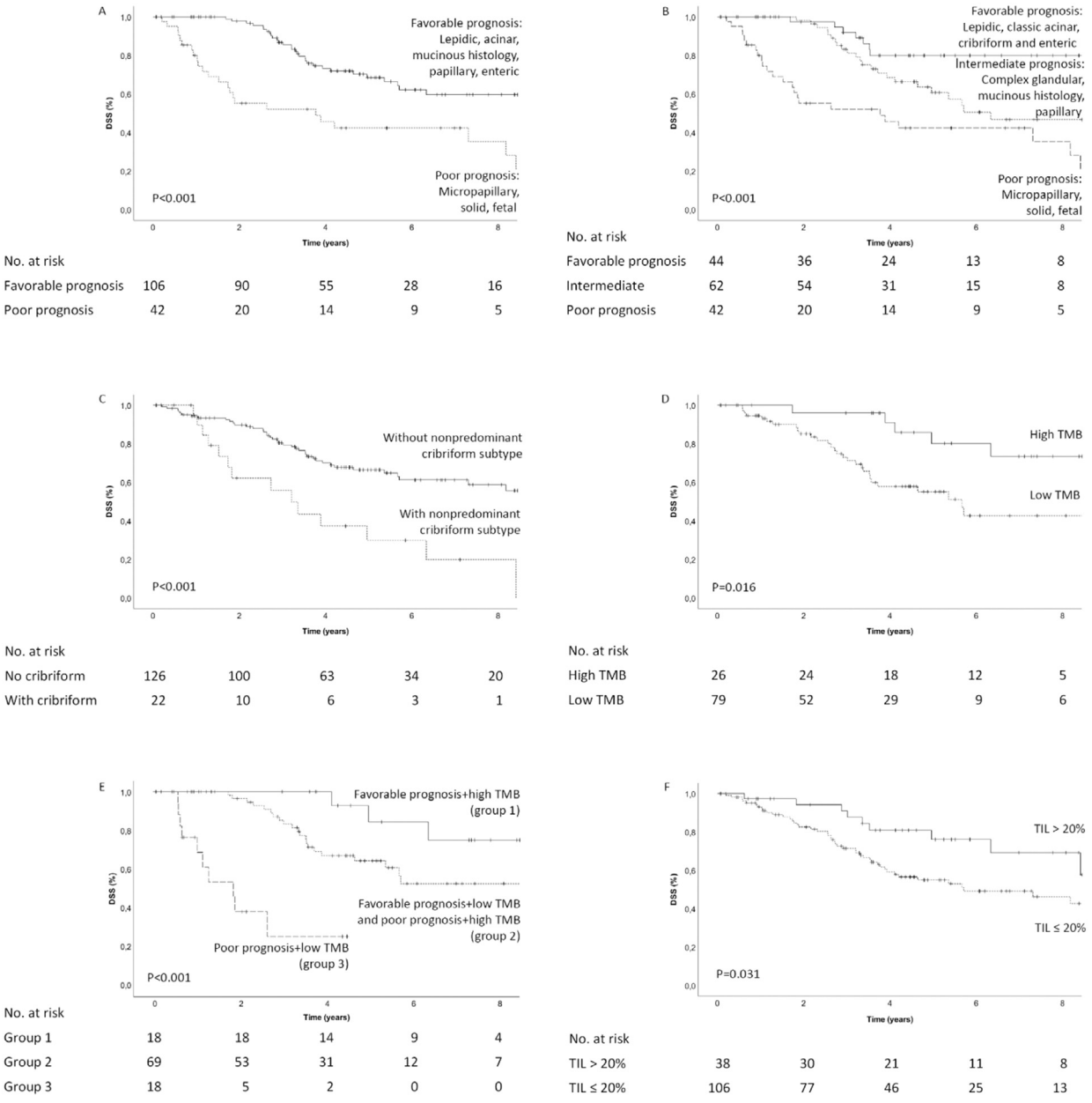

Fig. 3. The prognostic value of tumor characteristics on disease specific survival. Kaplan-Meier analysis demonstrating disease specific survival (DSS) for IASLC/ATS/ERS predominant subtypes (A), modified predominant subtypes (B), nonpredominant cribriform component (C), tumor mutation burden (TMB) (D), IASLC/ATS/ERS prognostic groups combined with TMB data (E) and abundance of tumor infiltrating lymphocytes (TILs) at the tumor invasive front (F).

growth patterns exhibiting a glandular appearance differed from this definition, presenting with jagged, branching, fused, or sieve-like glandular structures (Fig. 2). A few previous studies have suggested that predominant glandular patterns more complex than the IASLC/ATS/ERS description herald poor survival [5–10], and our results concur with these observations. In particular, predominant complex glandular subtype was associated with poor prognosis in our cohort when compared to classic acinar tumors in univariable analysis.

In spite of a relatively small number of tumors, high TMB at least partially ameliorated the dismal prognosis of the high-grade tumors in our cohort. This phenomenon was especially pronounced in cribriform predominant tumors, several of them harboring high TMB. Although nonpredominant cribriform subtype was associated with particularly poor survival, even in this group the few cases with high TMB had a trend towards longer survival (data not shown). As expected, all patients with high TMB tumors were smokers, a habit known to induce a high number of mutations [13].

Devarakonda et al. were the first to report improved prognosis among patients with high nonsynonymous TMB in non-small cell lung cancer (NSCLC), including adenocarcinoma [14]. In their analysis, a large targeted NGS panel and a TMB cutoff of >8 mutations/MB (the highest tertile) was used. By contrast, two recent studies by Owada-Ozaki et al. [15] and Wang et al. [16] associated high TMB with poor prognosis in NSCLC [15] or lung adenocarcinoma [16]. Owada-Ozaki et al. deter-

mined TMB by whole exome sequencing (WES) and defined high TMB as equal or more than 62, the median of TMB in their study. Wang et al. also used WES data with a cutoff of 163.5, the mean of TMB. Our results, also acquired using a targeted NGS panel, support the observation by Devarakonda et al. Wang et al. hypothesized that one possible explanation for these differences could be that different regions of genes were analyzed when determining the TMB. Thus, the results of NGS- and WES-based analyses may not be directly comparable in all cases. One should also bear in mind that the study populations of our and Devarakonda et al. were predominantly of Western origin while two other aforementioned studies included East Asian patients. Thus, the genetic differences between the ethnic populations may be one confounding factor. Nevertheless, the number of studies so far is too low to draw reliable conclusions between different analysis methods and prognosis, and further studies with both NGS- and WES-based approaches are needed.

TMB is thought to represent an estimate of the load of tumor neoantigens recognized by the immune system [21]. The evaluation of TILs on HE-stained slides has been shown to be of prognostic importance in NSCLC, with a higher density of TILs serving as a marker for good prognosis [22–24]. Devarakonda et al. hypothesized that the number of TILs would be correlated with TMB in NSCLC but this proved not to be the case [14]. Correspondingly in our study, the abundance of TILs at the tumor invasive front was not associated with TMB status even though high TIL density was associated with favorable prognosis in the univariable analysis. Although the sampling and selection of tumor slides may interfere with TILs or TMB analysis, this result suggests that factors other than TILs confer the prognostic effect of TMB.

The strength of the current study is comprehensive clinicopathological follow-up data with the most recent, updated, and re-reviewed staging and histopathological classification. The main limitations include the retrospective nature of the study, a relatively small cohort especially for prognostic evaluation of different subgroups, and the fact that the treatment practices of metastasized lung adenocarcinoma have changed during the duration of the study, possibly influencing survival. However, the latter was not supported by our data as there were no significant differences in given adjuvant therapies between the low and high TMB groups (Tables S1 and S2).

In conclusion, we showed that high TMB, as determined by a comprehensive targeted NGS panel, is a favorable prognostic biomarker in lung adenocarcinoma after surgery, especially in aggressive histological types. After further validation, TMB might be a useful tool for risk stratification in routine clinical practice.

Conflict of interest

Dr. Talvitie reports personal fees from Roche Oy, grants from Roche Oy, during the conduct of the study. Dr. Vilhonen reports grants from The Finnish Medical Foundation, grants from Finnish State Research Funding Grant, grants from Roche, during the conduct of the study; personal fees from Roche, personal fees and non-financial support from Takeda, personal fees and non-financial support from Boehringer Ingelheim, outside the submitted work. Dr. Singh was an employee of Roche Pharma Finland at the time of the conduction of the study. Dr. Liljeroos is an employee of Roche Oy and has equity interest in F. Hoffmann La Roche AG.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neo.2020.05.004.

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