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The value of prognostic and predictive parameters in early-stage lung adenocarcinomas: A comparison between biopsies and resections

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ABSTRACT

Introduction: Since lung adenocarcinoma (LUAD) biopsies are usually small, it is questionable if their prognostic and predictive information is comparable to what is offered by large resection specimens. This study compares LUAD biopsies and resection specimens for their ability to provide prognostic and predictive parameters.
Methods: We selected 187 biopsy specimens with stage I and II LUAD. In 123 cases, subsequent resection specimens were also available. All specimens were evaluated for growth pattern, nuclear grade, fibrosis, inflammation, and genomic alterations. Findings were compared using non-parametric testing for categorical variables. Model performance was assessed using the area under the curve for both biopsies and resection specimens, and overall (OS) and disease-free survival (DFS) was calculated.
Results: The overall growth pattern concordance between biopsies and resections was 73.9%. The dominant growth pattern correlated with OS and DFS in resected adenocarcinomas and for high-grade growth pattern in biopsies. Multivariate analysis of biopsy specimens revealed that T2-tumors, N1-status, *KRAS* mutations and a lack of other driver mutations were associated with poorer survival. Model performance using clinical, histological and genetic data from biopsy specimens for predicting OS and DSF demonstrated an AUC of 0.72 and 0.69, respectively.
Conclusions: Our data demonstrated the prognostic relevance of a high-grade growth pattern in biopsy specimens of LUAD. Combining clinical, histological and genetic information in one model demonstrated a suboptimal performance for DFS prediction and good performance for OS prediction. However, for daily practice, more robust (bio)markers are required to predict prognosis and stratify patients for therapy and follow-up.

1. Introduction

The standard treatment for small, non-metastatic lung adenocarcinoma (LUAD), regardless of morphology or mutational status, remains complete surgical resection by means of lobectomy with hilar and mediastinal lymph node dissection [1] or (stereotactic) radiotherapy (RT), especially if contraindications are present (such as poor lung function, comorbidity or patient preference) [2]. However, there are indications that sublobar resections (by wedge excision or segmentectomy) may provide similar long-term outcomes in low-risk LUAD, which is a preferable option to preserve lung function and limit treatment-associated morbidity [3,4]. This is especially important in those

Abbreviations: LUAD, lung adenocarcinoma; RT, radiotherapy; SUV, standardized volume uptake; PET, positron emission tomography; IASLC, International Association of Lung Cancer; ATS, American Thoracic Society; ERS, European Respiratory Society; WHO, World Health Organization; OS, overall survival; DFS, disease-free survival; CT, computer tomography; (p)TNM, (pathological) tumor node metastasis; NGS, Next-generation sequencing; CI, confidence interval; ROC, receiver operating curve.

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patients with limited lung function and in view of the fact that LUAD frequently presents with separate synchronous or metachronous tumors in different, sometimes contralateral lobes of the lung. Up to now, the choice for a lung sparing approach mainly depends on radiologic appearance such as the size of the nodule or the standardized volume uptake (SUV) when using positron emission tomography (PET) [4].

Since the introduction of the LUAD growth patterns as defined by the International Association of Lung Cancer (IASLC), American Thoracic Society (ATS) and European Respiratory Society (ERS) in 2011 [5], and recently introduced into the World Health Organization (WHO) classification [6], many studies have evaluated their prognostic value in resected specimens [7–9]. Next to the growth pattern, other histological parameters such as nuclear grade, mitotic activity [10,11], fibrosis [12,13], and genetic alternations have been evaluated with regard to their prognostic value [14]. So far, however, no combinations of the above-named factors have been described in the literature for prognostication of early-stage LUAD in biopsy specimens.

We hypothesized that the predictive value of the pre-operative assessment of LUAD can be increased by combining (1) clinical information with (2) biopsy histology, and (3) mutation analysis, and should be taken into account when selecting patients for a given surgical or radio-therapeutical approach.

To investigate this hypothesis, we first evaluated the predictive value of the histomorphology in resected LUAD and compared it with the preoperative biopsy. Second, we correlated these parameters with overall survival (OS) and disease-free survival (DFS) for both resection and biopsy specimens. Third, we developed a model which best predicts OS and DFS in patients with lung cancer who underwent a pre-operative biopsy by combination of clinical, histological and genetic data.

2. Material and methods

2.1. Study population

We evaluated a single-center retrospective series of all 7,226 patients who had undergone a lung biopsy in our institution between January 2009 and December 2018. Of these patients, 367 were diagnosed with LUAD. Biopsies were obtained by transbronchial biopsy or by computer tomography (CT)-guided transcutaneous needle biopsy. Specimens other than adenocarcinoma, cT3 and cT4 tumors, multiple lung nodules, cN2 stage, M1 disease, wedge resections/lobectomies without preoperative biopsy samples, and those with missing clinical data, were excluded. Following these criteria, 187 samples of patients were included in this study. For the flow diagram of the selection process, see Supplemental Fig. A1.

All patients had undergone a lung biopsy to confirm the diagnosis of LUAD. In the course of time, 126 of the 187 patients underwent a lobectomy or wedge resection, of which 123 were performed in our institution and available for analysis.

2.2. Review of hematoxylin-eosin (HE) stained preoperative small biopsies and postoperative resection specimens

HE sections of preoperative lung biopsies and postoperative resection specimens were independently reviewed by two pathologists (JT and JW), who were blinded to the pathology reports as well as the clinical data. The IASLC/ATS/ERS classification [15] was applied, using 5 % increments for the proportions of growth patterns present in the specimens. Tumors were divided into lepidic, acinar, papillary, micropapillary and solid classes based on the dominant growth pattern present. For resection specimens, tumors were also classified as adenocarcinoma in situ and minimally invasive adenocarcinoma according to the IASLC/ATS/ERS classification. Besides determining the predominant growth pattern on biopsies and resection specimens, nuclear grading, classification of the desmoplastic reaction and inflammation within the tumor were recorded in biopsies and resection specimens following a categorical definition of these parameters. For specific grading schemes, see Supplemental Table A1–A3.

For all resection specimens, we recorded the eighth version of the pTNM stage. Tumors diagnosed with pTNM7 were reclassified into pTNM8. The size of the invasive component was measured, and visceral pleural invasion was recorded. If available, Verhoeff van Gieson staining for elastic fibers was used to determine pleural invasion.

Discrepancies concerning the histological findings were in a second step resolved by consensus at a double-headed microscope.

2.3. Mutation analysis

Diagnostic targeted DNA next-generation sequencing (NGS) for mutation detection (including point mutations, deletions and insertions) and in case of absence of DNA alterations, gene fusion/rearrangement profiling by fluorescence in situ hybridization (FISH) analysis and immunohistochemistry, was initially performed on 148 biopsies (96.1 %) and 6 resections (3.9 %). In two cases (1.3 %) with insufficient material in the biopsy, molecular testing was instead performed in one case on cytology, and in the other case on the corresponding resection specimen. In 8 cases (5.2 %), complete genetic testing was performed on both the primary and the resection. In brief, a targeted NGS panel, used in the routine diagnosis of lung malignancies and comprising more than 50 commonly mutated genes (including hot-spot analysis of *EGFR*, *KRAS*, *MET*, *BRAF, and HER-2*, and full-length exonal analysis of *P53*), was performed on either the biopsy or the resection specimen, as described previously in the Supplementary data of Hermelijn et al. [16]. If the NGS analysis did not reveal a driver alteration, the tissue underwent translocation and amplification testing by either Archer® FusionPlex Lung panel Targeted RNA Sequencing or by FISH analysis for ALK, RET en ROS1 rearrangements and MET amplifications, as well as in all cases immunohistochemical screening for ALK fusions.

2.4. Statistical analyses

2.4.1. Associations between categorical data

The clinicopathological characteristics of the patients are reported descriptively, whereas data are presented as numbers or ratios (%). All statistical analyses were performed using R version 4.0.2.

Relationships of two categorial variables were visualized using contingency tables. Fisher's exact test was chosen when (row total*-column total)/total sample size < 5. For a sample size greater than 5 Pearson's Chi-squared test with Yates' continuity correction was used. Data analysis was performed using the R-packages "gmodels" (R version 4.0.3) and "summary tools" (R version 4.0.5).

Circos plots that illustrate changes in relationships between two categories were created using R "circlize" package version 0.4.10. Pie charts were plotted with the "ggplot2" package. (R version 4.0.3).

2.4.2. Survival analysis

Survival curves with confidence intervals (CI) and numbers at risk (i.e. death or recurrence) were analyzed separately for the biopsy and resection group using Kaplan Meier methods. OS was defined from time of biopsy until death from any cause. Patients who remained alive at the time of the last follow-up were censored. DFS was defined from time of biopsy until recurrence. Patients who remained alive or did not present with recurrence at the time of the last follow-up were censored. Correction for multiple testing was not performed.

Univariate Cox regression analysis was performed using the R packages "survival", "ggplot2", "ggpubr" and "survminer" (All R Version 4.0.3.). The covariates age, sex, smoking state, *T*-stage, *N*-stage clinical-stage, dominant growth pattern, fibrosis, nuclear grading, inflammation, driver mutations and presence/absence of p53 mutation were used. Within the dataset, 73 events occurred. Covariate estimates which showed a p-value < 0.2 within the univariate analyses were entered into the multivariate analysis. The Schoenfeld residuals were used to check

time dependency of the covariates. There was a non-significant relationship between residuals and time. P-values < 0.05 in the multivariate analysis were considered to be significant.

Receiver operating curve (ROC) analyses were performed to test the predictive performance of the given clinical, histological and genetic data for OS and DFS in biopsy and resection specimens. Therefore, the dataset was randomized and divided at random into a training (n = 140) and a validation (n = 47) set. Fourfold cross-validation was done to test the performance of the model, and the highest AUC per training set was chosen. The "ROCR" package (R-version 4.0.5) was used for analysis.

The institutional review board approved this study and waived informed consent (MEC-2020–0732).

3. Results

3.1. Patient characteristics

Ultimately, 187 patients were selected for the study, including 107 males and 80 females (mean age 72 years). Within this cohort, 157 patients had a history of smoking. 126 patients received surgery, 53 underwent radiotherapy and 8 patients received no therapy. Baseline characteristics are summarized in Table 1.

3.2. Histologic features of lung biopsies

The most prevalent dominant growth pattern observed in a biopsy specimen was lepidic (39.0 %), followed by acinar (38.2 %) and solid (20.3 %). Papillary and micropapillary growth pattern were rare, at 1.6 % and 0.8 % respectively.

We first determined the relationships between the dominant growth pattern and nuclear grade, inflammation and fibrosis.

Lepidic tumors significantly correlated with low and intermediate nuclear grade. The majority of acinar tumors (50.5 %) had an intermediate nuclear grade followed by high-grade morphology. Solid tumors more often presented with a high-grade nuclear pattern. The four cases with papillary growth correlated with low nuclear grade.

The majority of lepidic tumors showed a good correlation with little to moderate fibrosis. Absence of fibrosis was observed in 11 cases, high fibrosis was noted in only 6 cases. In most acinar dominant tumors, moderate fibrosis was present, while none lacked fibrosis. In the solid group, minor and high fibrosis were observed.

No correlations were found between the number of inflammatory cells and the dominant growth pattern. For details, see Supplemental Fig. A2.

Table 1
Clinicopathological features of the study population.

Characteristic	N = 187
Gender	
Male	107 (57.2 %)
Female	80 (42.8 %)
Age (median, range)	72 (38–89)
Smoking status	
Smoker	81 (43.0 %)
Former smoker	76 (41.0 %)
Not a smoker	30 (16.0 %)
Diagnostic procedure	
Computed tomography guided biopsy	175 (94 %)
Endobronchial biopsy	10 (5.0 %)
Unknown	2 (1.0 %)
Survival status	
Alive	113 (60,4%)
Death	74 (39.6 %)
Therapy	
Radiotherapy	53 (28.3 %)
Surgery	126 (67.4 %)
No therapy	8 (4.3 %)
Tumor size (mm)	20 (7–56)
TNM classification	
T1a	10 (5,3%)
T1b	89 (47,6%)
T1c	52 (27,8%)
T2a	25 (13.4 %)
T2b	11 (5,9%)
N0	176 (94.0 %)
N1	11 (6.0 %)
Clinical stage	
1A1	10 (5,3%)
1A2	86 (46,0%)
1A3	48 (25,7%)
1B	22 (11.8 %)
2A	13 (5,3%)
2B	11 (5,9%)

3.3. Mutation data

In the cohort, 154 cases (82.4 %) had undergone targeted genetic testing. 108 cases (57.75 %) showed a driver mutation, while 34 tumors (18.2 %) did not reveal a driver mutation and 12 cases (6.4 %) did not display any mutation. *KRAS* was the most frequently mutated gene. Supplemental Fig. A3 shows the distribution of the different driver alterations. Additionally, 70 of these 154 cases (45.45 %) presented with a *TP53* (co–) mutation. In 8 cases (5.2 %), complete genetic testing was performed on both the primary biopsy and the resection. This identified identical mutations, except in one case, in which additional *PIK3CA* and *TERT* mutations were found in the resection specimen.

There was a strong correlation between *EGFR* mutations and tumors with a dominant lepidic growth pattern ($\chi^2(1) = 7.37$, $p = 0.006$). In addition, lepidic growth correlated with *MET* exon 14 skipping mutations ($\chi^2(1) = 4.96$, $p = 0.026$). By contrast, tumors with solid growth relatively lacked driver mutations ($\chi^2(1) = 9.63$, $p = 0.001$). No other significant correlations between dominant growth pattern and mutation status were present.

3.4. Comparison of histological features between lung biopsies and resection specimens

There was a fair concordance rate of the dominant growth pattern of 73.9 % between biopsies and resection specimens. The strongest concordance was shown for the acinar growth pattern (89.3 %), while the lowest concordance rates were found for micropapillary growth. In the solid subgroup, a concordance rate of 72 % was observed, in the lepidic tumors the concordance rate was 62.5 %. There was a strong correlation seen between the dominant patterns present in the biopsy with those in the resection specimen (See Supplemental Table A4). In the lepidic subgroup, we observed a change to an acinar pattern in 14 cases, to micropapillary in three and to solid and papillary in one case. Furthermore, we observed two cases of an acinar- to a solid-dominant pattern change (Fig. 1). When focusing on the worst and prognostically most relevant growth pattern in the biopsy (i.e. solid and micropapillary), even when present as a minor growth pattern, the concordance rate was less accurate in the resection specimen. The relationships for growth patterns between the biopsy and the resection specimen and potential shifts are visualized in Fig. 1.

The nuclear grade, fibrosis and inflammation relationships between biopsy and resection specimen were evaluated (See Supplemental Table S5). When analyzing and correlating the nuclear grade between biopsy and resection specimen, we observed a shift towards a higher nuclear grade in the resection specimen. This was predominantly present for the moderate nuclear grade in the biopsy, which presented more often as a high-grade pattern in the resection specimen. To a lesser extent, the same was observed for biopsy specimens with a low nuclear grade.

Similar observations were drawn from the quantification of fibrosis, where no fibrosis eventually became little fibrosis, little fibrosis became moderate and moderate became high fibrosis in resection specimens.

The shift towards a higher inflammatory infiltrate in the resection

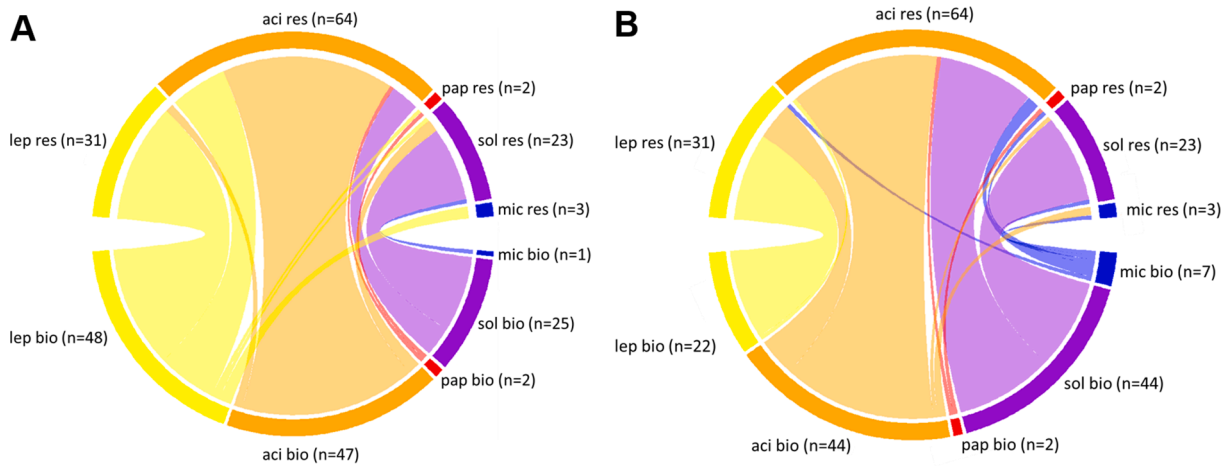

Fig. 1. (A) Relationship of the dominant growth pattern between biopsy (lower half of the circle) and resection specimen (upper half of the circle). Connections are marked in the color of the biopsy. (B): Relationship of the growth pattern with the worst prognosis (solid and micropapillary) between biopsy (lower half of the circle) and dominant pattern of the resection specimen (upper half of the circle). Any presence of the worst pattern was used even when present as a minor component. Connections are marked in the color of the biopsy. (lep = lepidic, *aci* = acinar, pap = papillary, mic = micropapillary, sol = solid, bio = biopsy, res = resection).

specimens compared to the biopsies was, on the other hand, less prominent.

3.5. Survival analysis using Kaplan Meier method

Survival analysis using the Kaplan Meier method was performed for the histological parameters of growth pattern, nuclear grade, fibrosis and mutational state. The median follow-up was 2.89 years, 95 % CI [0.36,5.42]. Median OS was 2.092 years, 95 % CI [0.11,4.29] and median DFS was 1.45 years, 95 % CI [0.5,3.37]. The five different histopathological growth pattern lepidic, acinar, papillary, micropapillary and solid were combined into a low-grade (i.e., lepidic), moderate-grade (i.e., acinar and papillary) and high-grade pattern (i.e., micropapillary and solid). Survival analysis (for OS and DFS) of the 123 resected specimens showed a clear separation of the survival curves. In resected LUAD, patients who presented with a low- and moderate growth pattern had a lower risk to die from or recur with LUAD compared to those patients who presented with a high-grade growth pattern (HR moderate-grade growth pattern for OS: 0.51, 95 %CI [0.25,1.02] and DFS: 0.32, 95 %CI [0.15,0.68]; HR low-grade growth pattern for OS: 0.1, 95 %CI [0.02,0.45] and DFS: 0, 95 %CI [0.00, Inf]). This was also true for the 186 biopsies, although no stratification between a low- and moderate-grade growth pattern was evident (Fig. 2A–D).

In a second step, survival analysis was performed for the nuclear grade and carried out separately for biopsies and resection specimens. In resected LUAD, patients who presented with low and moderate nuclear grade had a lower risk to die from or recur with LUAD compared to those patients who presented with a high nuclear grade (HR moderate nuclear grade for OS: 0.31, 95 %CI [0.12,0.80] and DFS: 0.42, 95 %CI [0.16,1.11]; HR low nuclear grade for OS: 0.13, 95 %CI [0.03,0.55] and DFS: 0,09, 95 %CI [0.01, 0.64]) (Fig. 2E–H). Survival analysis was also performed for the amount of fibrosis. However, no significant findings in biopsy and resection specimens were observed for both OS and PFS.

Patients who presented with an absence of driver alterations or who harbored a *KRAS* mutation had a higher risk to die from early stage LUAD compared to those who presented with an *EGFR* mutation (HR absence of driver mutation for OS: 4.09, 95 %CI [1.61,10.4] and DFS: 2.25, 95 %CI [0.85,5.93] and HR KRAS for OS: 2.28, 95 %CI [0.92,5.69] and DFS 1.71, 95 %CI [0.71,4.14]). Patients with a *TP53* co-mutation showed a better OS and DFS compared to those who lacked a mutation in this tumor suppressor gene (HR TP53 for OS: 2.17, 95 %CI [1.26–3.75] and DFS: 2.05, 95 %CI [1.10,3.79]; see Supplemental Fig. A4).

3.6. Uni- and multivariate survival analysis

For all biopsies, the clinical covariates age, sex, smoking behavior, T-, N- and clinical stage were used together with the histological parameters defined by the growth patterns, fibrosis, nuclear grading and inflammation, as well as presence or absence of driver mutations, for univariate and multivariate Cox regression. Within the dataset, 73 events (deaths) occurred. Univariate analysis identified age, sex, *T*-stage, *N*-stage, clinical-stage, growth pattern, nuclear grading, driver mutations and presence of a *TP53* mutation as significant factors ($p < 0.2$). These covariates were subsequently used for multivariate analysis from which T2-tumor, N1-state, presence of a *KRAS* mutation and absence of a driver mutation were associated with worse survival and remained significant (see Fig. 3).

3.7. Survival and growth pattern prediction based on biopsy evaluation

Based on the previous findings of the uni- and multivariate analysis, we developed a ROC model to predict OS and DFS for patients with complete diagnostic processing using preoperative biopsy specimens and then compared the model performance with that of the resection specimens. In designing prediction models, various combinations of clinical, histological and genetic parameters were used, consisting of sex, age (less/greater than 75), smoking state, T/N stage, clinical-stage, dominant growth pattern, fibrosis, inflammation, nuclear grade, driver alterations in *EGFR*, *KRAS*, *BRAF*, *ALK*, *ROS*, *RET*, *MET* and *HER-2*, and the *TP53* state. The first model was based on the significant parameters from the univariate analysis and showed in the biopsy subgroup an AUC of 0.52 and 0.68 for recurrence and death, respectively, which increased in the resection specimens to 0.79 and 0.71, respectively (Table 2 Model No 1). Since the presence or absence of different growth patterns is of prognostic importance, different combinations of those were used together with or without clinical parameters to increase the model performance in a second step. When using a combination of the dominant- and the worst growth pattern with a cut-off of 20 %, the model performance in the biopsy subgroup for predicting recurrence increased to 0.72 (Fig. 4A Model No 7). For predicting death on biopsy specimens, the parameters of the multivariate analysis remained the best model (Fig. 4B). Similar performance was also observed for the recurrence prediction in resection specimens (Fig. 4C). On the other hand, model

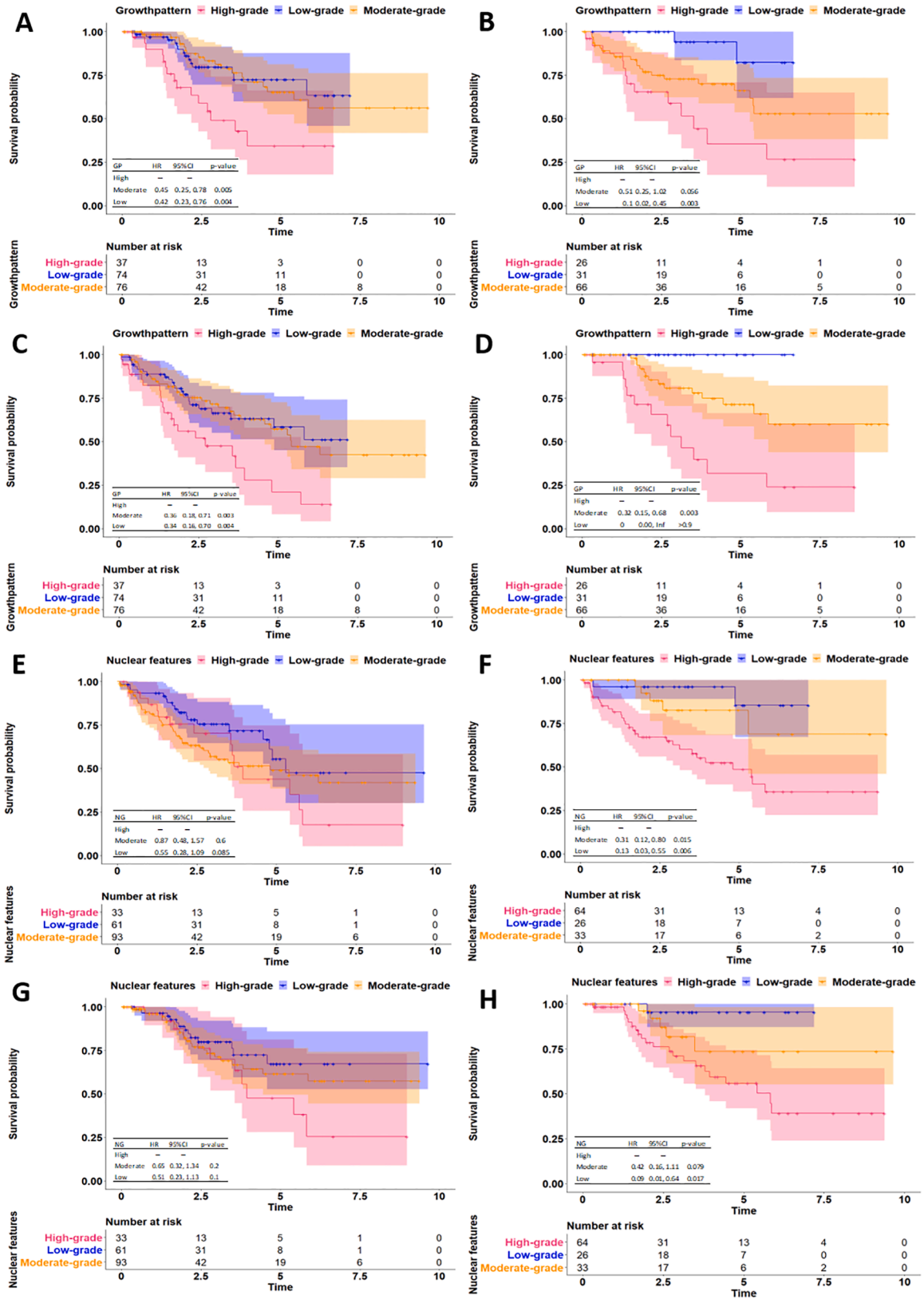

Fig. 2. OS stratified according to the growth pattern for biopsies (A) and resection specimens (B). DFS of the respective growth pattern for biopsies (C) and resection specimens (D). The growth pattern stated on the resection specimen showed a better prognostic course compared to the biopsy. OS stratified according to nuclear grade for biopsies (E) and resection specimens (F). DFS stratified (Disease Free Survival) stratified according to nuclear grade for biopsies (G) and resection specimens (H). The nuclear grade in resection specimens shows a better prognostic course compared to biopsies. (OS = overall survival, DFS = disease free survival, GP = growth pattern, NG = nuclear grade, HR = Hazard ratio, Time = Time in years).

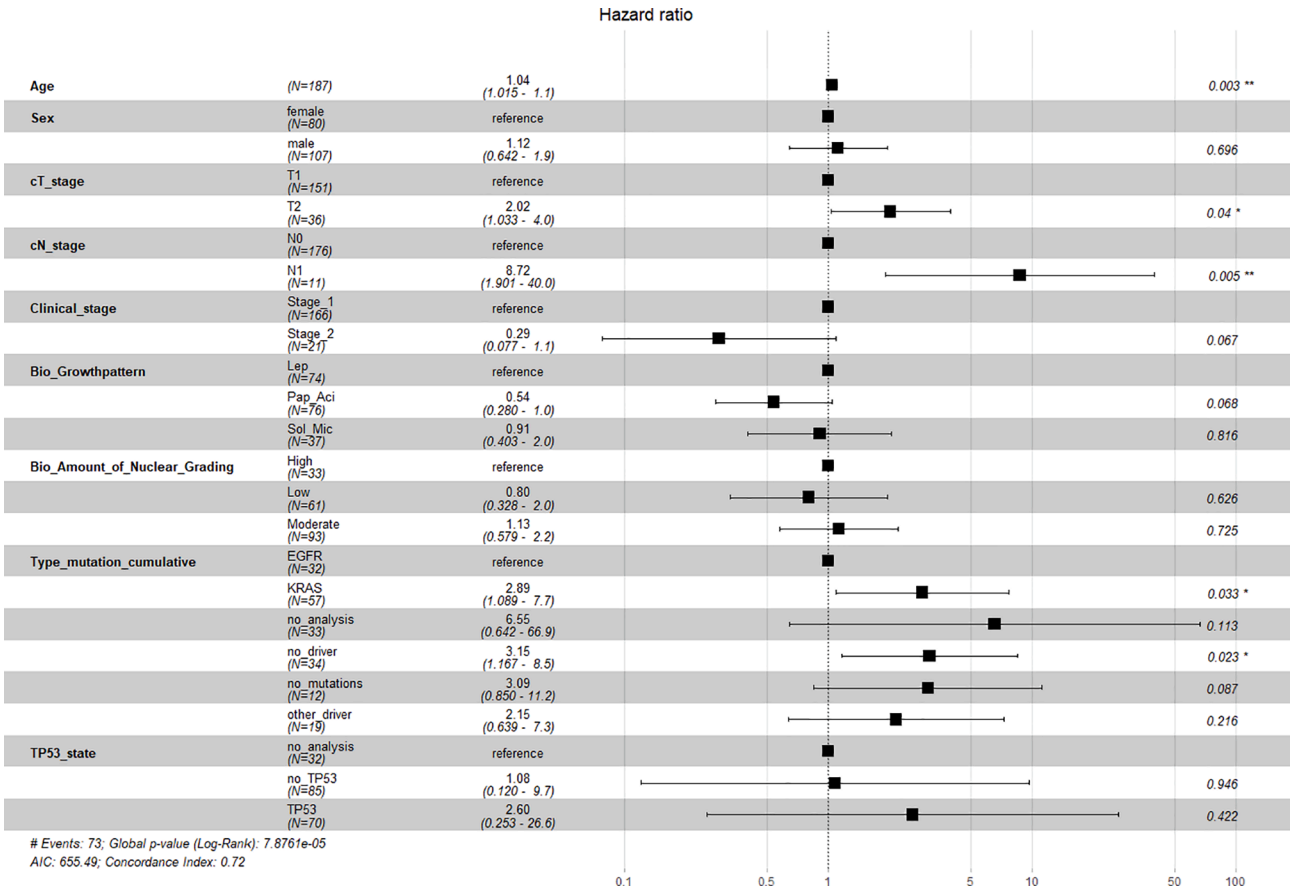

Fig. 3. Multivariate analysis after selection of the significant parameters in univariate survival analysis. Significant parameters from univariate analysis were gender (sex), clinical tumor (cT) and nodal (cN) stage, the clinical stage, the growth pattern, nuclear features, mutational state and TP53 state of all available biopsies (Lep = lepidic, *Aci* = acinar, Pap = papillary, Sol = solid, Mic = micropapillary, Bio = biopsy).

Table 2
Results of ROC analysis for biopsies (left) and resection specimens (right) for predicting recurrence and death. Tested was in first instance the performance of the multivariable analysis. To increase model performance, various combinations of reporting growth pattern were tested with and without clincial information. (Model No. = number of models tested, Dom = Dominant, GP = Growth pattern, Clin = including clinical data, Histo = histology parameters only, Cont = continuous; the percentages of the given growthpattern were entered in the model, Cum = cumulative; growthpattern were summarized into low, intermediate and high grade, 2nd = second dominant growth pattern, worst = worst growth pattern, worst5/20/35 = worst growth pattern with a cut-off of 5 %/20 %/35 %.).

		Biopsy				Resection			
		Recurrence		Death		Recurrence		Death	
Model No.	Variables in the model	Clin	Histo	Clin	Histo	Clin	Histo	Clin	Histo
1	Multivariable_Analysis	0.526		0.689		0.798		0.719	
2	DomGP	0.617	0.695	0.609	0.577	0.748	0.695	0.752	0.640
3	ContGP	0.597	0.695	0.638	0.575	0.671	0.648	0.671	0.657
4	CumGP	0.565	0.617	0.617	0.573	0.724	0.701	0.757	0.783
5	DomGP + 2nd_GP	0.556	0.630	0.646	0.593	0.672	0.645	0.690	0.633
6	DomGP + worst5	0.589	0.707	0.626	0.552	0.727	0.738	0.642	0.657
7	DomGP + worst20	0.642	0.723	0.624	0.577	0.674	0.706	0.690	0.714
8	DomGP + worst35	0.580	0.638	0.621	0.582	0.682	0.716	0.723	0.623
9	DomGP + 2nd + worst5	0.630	0.626	0.631	0.618	0.738	0.677	0.868	0.654
10	DomGP + 2nd + worst20	0.570	0.617	0.64	0.605	0.658	0.663	0.685	0.633

performance for predicting death on resected specimens could be improved up to 0.86 when using a combination of the clinical features together with the dominant, the second dominant and the worst growth pattern with a cut-off of 5 % (Fig. 4D, Model No 9). We further observed that recurrence prediction worked best on histology data compared to predicting death, where model improvement could be observed after adding clinical information.

4. Discussion

This study aimed to demonstrate the importance and relevance of the histological and mutation analysis of biopsy specimens in determining a patient's outcome and clinical decision-making in early-stage LUAD. To this end, we analyzed a dataset of 187 patients who underwent a diagnostic biopsy prior to surgical or radiotherapeutic intervention and related growth pattern, nuclear grade, fibrosis, inflammation and available genetic data, with patient outcome. These parameters were

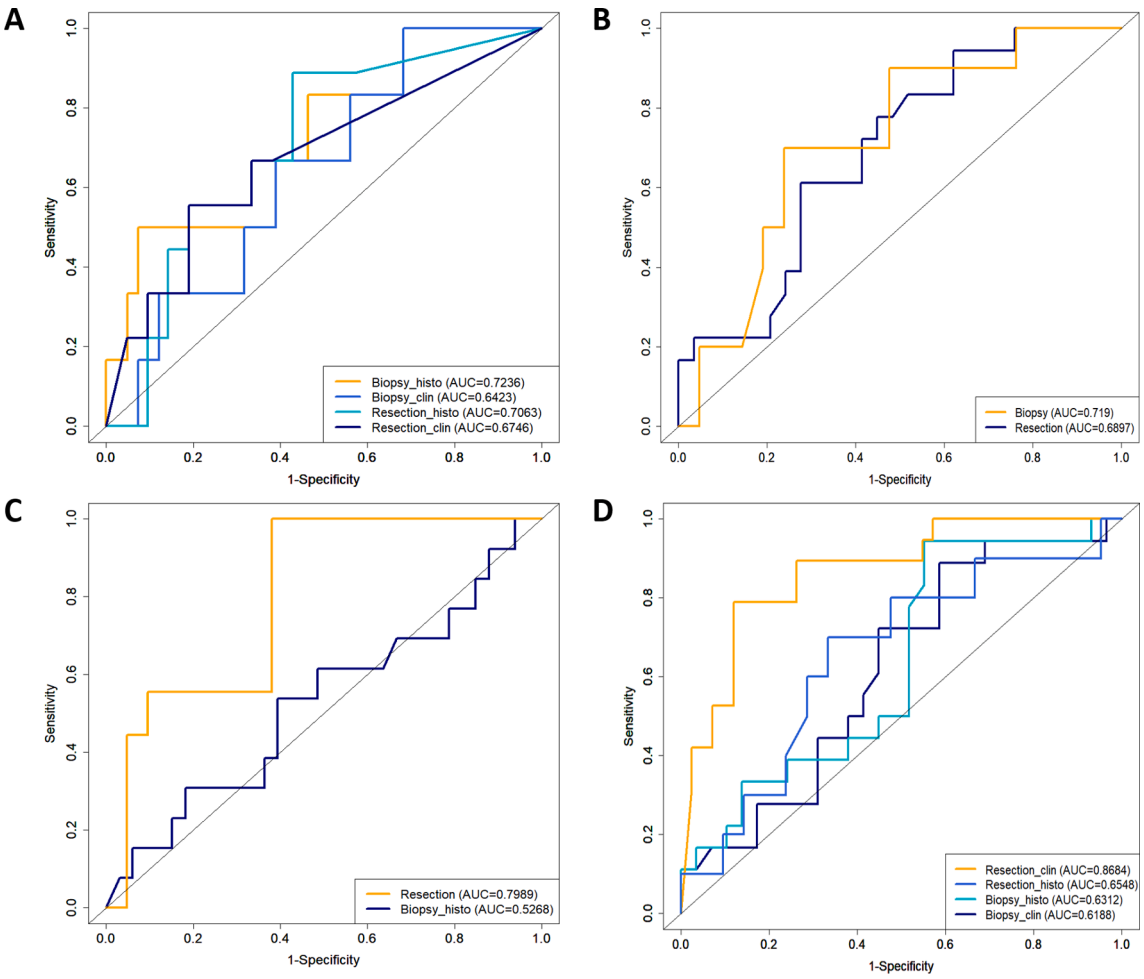

Fig. 4. Visualization of model performance per category (A) Recurrence prediction for biopsies using a combination of the dominant and high-grade growth pattern with a cut-off of 20 percent. (Model No. 7) (B) Death prediction for biopsies using the data of the multivariable analysis. (Model No. 1) (C) Recurrence prediction for resection specimens using the data of the multivariable analysis. (Model No. 1) (D) Death prediction for resection specimens using a combination of the dominant, second dominant and high-grade growth pattern with a cut-off of 5 %. (Model No. 9) (orange line: best performing model per category, blue lines: remaining model performances per category for comparison; clin = clinical data, histo = histological data only, AUC = area under the curve). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

then compared with findings in a sub-cohort who also underwent a surgical resection to make a statement about the prognostic value between both modalities.

The prognostic relevance of histological features such as growth pattern [7–9,17–19] nuclear grading [10,11,20], fibrosis [12,21–23] and inflammation [24] as well as genetic characteristics [14,25–28] have all been evaluated in resected specimens. To our knowledge no data on their value in biopsy specimens exists.

Therefore, we analyzed the prognostic value of biopsies and resection specimens according to the above-described features. The biopsy subgroup consisted of patients who underwent a resection and those who either received RT or no therapy. This approach was chosen instead of matched pairs to better represent early stage LUAD patients who undergo a diagnostic biopsy. Since RT is comparable to surgery in terms of survival [2], in our cohort not only patients with a poor performance were eligible for this modality.

For the biopsies, the high-grade pattern was accompanied by worse survival compared to low- and moderate-grade. This contrasts with the findings in resection specimens where the survival curves showed a clear separation between the low-, moderate- and high-grade growth pattern. Since patients selected for RT might present with a poorer outcome, subgroup analysis on the distribution of growth pattern was performed. The distribution of the growth pattern was equal in both cohorts; however, survival curves did not clearly separate for the various growth patterns (data not shown). To investigate these discrepancies, we analyzed the relationships of the different growth patterns between the preoperative biopsy specimen and the corresponding resections using matched pairs only. The presence of an acinar and solid predominant growth pattern in the biopsy was reproducible in the resection specimen. On the other hand, for lepidic tumors, we observed a shift towards a higher-grade pattern such as an acinar pattern in 14 cases, micropapillary in three cases and solid and papillary in one case. In addition, we observed a change of the acinar subgroup to solid dominant tumors in two cases. A reason for this observation might be tumor heterogeneity combined with sampling error when for example the biopsy is taken at the edge of the tumor, where lepidic growth is more often observed. This might also explain why the survival curves did not separate for low- and intermediate-grade tumors in the biopsy group. Furthermore, adding the RT patients to the analysis might slightly amplify this phenomenon.

When looking at the different growth patterns between biopsy and resection specimens, we reached an overall concordance for the predominant pattern of 73.6 % for all available pairs. The concordance observed is somewhat higher than Huang et al. stated in their study. They described a limited accuracy in predicting the predominant pattern between biopsy and resection specimens of only 58.6 %. [29] Reasons for differences observed in both studies might be the technique used for

extraction of material. In our study, more CT-guided biopsies were available with sufficient tumor material for adequate classification. In only 5 % of the cases an endobronchial biopsy was taken. As endobronchial biopsies are more often damaged due to crush artefacts, a reliable evaluation of the growth pattern might be limited. Furthermore, both datasets differ, insofar as the data from Huang et al. consisted of tumors derived from all stages whereas our dataset harbored only stage I and II tumors. They stated predominantly to observe problems with predicting a worse pattern, such as solid and micropapillary, which can be more often expected in advanced tumors. Our dataset contained insufficient cases with micropapillary growth pattern to draw significant conclusions. Nevertheless, the concordance of solid patterns between biopsies and resections was satisfactory with 73.1 %. Matsuzawa et al. found a lower concordance rate compared to our dataset (66 % vs 73.6 %). They found a higher concordance between preoperative biopsy specimens and corresponding resection specimens when the tumor increased in size. [30] This contradicts the chance of a greater sampling error in larger tumors when the specimen is not taken from the center of the mass.

When analyzing the biopsy specimens regarding growth pattern and the histological parameters nuclear grade and fibrosis, we observed several correlations. Thus, morphologically high-grade tumors were more likely to present with a higher nuclear grade compared to low-grade tumors. For the amount of fibrosis, the difference was less clear. Especially high-grade LUAD conspicuously often showed absence of fibrosis. When comparing the findings with the resection cohort, an upgrade of both parameters could be observed, and considering survival, only nuclear grade was of influence in resected specimens. Again, this might be a result of sampling error and tumor heterogeneity and a good explanation for the failure of the correlation with OS and DFS in biopsy specimens.

In the mutation analysis, biopsy specimens offered sufficient material in 98.7 % of our cases. In 8 cases for which genetic testing was performed on both the biopsy and the resection, identical driver and non-driver mutations were observed, except in one case in which the resection material also presented *PIK3CA* and *TERT* mutations. This can be explained by tumor heterogeneity when performing analysis on a different tumor area. However, driver mutations such as *KRAS* as well as the *TP53* state, which were significant in multivariate analysis for survival, were detected in both biopsy and resection. We can thus state that early mutation testing on biopsies offers results which can reliably influence therapeutic decision making in early-stage LUAD, including predictive testing for (neo)adjuvant therapy or prognostic evaluation.

Combining clinical information with histological findings and available genetic data, we developed an ROC model on biopsy specimens to predict OS and DFS of patients presenting with early-stage pulmonary adenocarcinoma. When using the significant parameters from the univariate analysis, model performance was poor to moderate for predicting DFS and OS in biopsies (with an AUC of 0.52 and 0.68 respectively) and moderate for the resections (AUC = 0.79 and 0.71). To increase model performance we focused on different growth pattern combinations together with or without clinical information as performed by Moreira et al. for resected tumors [31]. This led to an increase in model performance with an AUC of 0.72 and 0.69 for DFS and OS in the biopsies and an AUC of 0.79 and 0.86 in the resections. The findings are comparable to those of Moreira et al. who reached an AUC of 0.76 for DFS when analyzing the dominant and second dominant pattern and an AUC of 0.78 for OS when focusing on the dominant and worst pattern with a cut off of 20 % [31]. The slight differences in model performance might be explained by the fact that our model also included additional histological parameters such as nuclear grade, fibrosis and inflammation as well as genetic data. Furthermore, clinical information differs between both datasets. It is not surprising that model performance is higher for the resected subgroup, since more tissue is available for analysis and this is in line with the findings of the Kaplan Meier curves.

5. Conclusion

In conclusion, we state that the evaluation of the combination of clinical parameters, morphology and mutation status of preoperative LUAD biopsy specimens can predict, to some extent, a patient's prognosis and are an important addition to cTNM which is already known as a prognostic factor. In our dataset we observed that the growth pattern seemed to have a higher impact on a patient's prognosis compared to the cTNM classification and the clinical stage. This might be explainable by the presence of only low-stage patients in our cohort but might also be caused by the population selection (regarding the histological types of T1 tumors included). Therefore, the information derived from the biopsy, such as growth pattern, nuclear grade, amount of fibrosis and mutation status can next to the clinical information be a valuable addition in selecting patients for RT or defining the extent of a surgical procedure. Furthermore, histomorphology together with PD-L1 analysis and genetic testing in biopsies can be used in the future to better predict the response to neoadjuvant therapy. In addition, genetic testing of LUADs is becoming increasingly implemented in an adjuvant setting. For example, adjuvant atezolizumab has recently been approved for tumors with a PD-L1 expression of more than 50 % in resected stage II-IIIa tumors [32], and Osimertinib for *EGFR* mutant tumors [33].

Further studies to validate our findings and model using larger cohorts and methods are needed to extract more robust predictive parameters from biopsy specimens to better guide clinical decision-making for early-stage lung cancer and to evaluate prognosis. In addition, development of an artificial intelligence model to predict recurrence may help in stratifying patients for therapy.

Summary conflict of interest statement

The authors Janina Wolf, Teodora Trandafir, Farhan Akram, Elrozy Andrinopoulou, Andrew Stubbs, Johan Max Kros, Alexander (Lex) Maat and Dana Mustafa declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work of this paper.

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CRediT authorship contribution statement

J.L. Wolf: Conceptualization, Methodology, Software, Formal analysis, Investigation, Resources, Visualization, Writing – original draft. **T. E. Trandafir:** Software, Visualization, Writing – review & editing. **F. Akram:** Software, Visualization, Writing – review & editing. **E.R. Andrinopoulou:** Formal analysis, Software, Writing – review & editing. **A.W.P.M. Maat:** Writing – review & editing. **D.A.M. Mustafa:** Writing – review & editing. **J.M. Kros:** Writing – review & editing. **A.P. Stubbs:** Writing – review & editing. **A.C. Dingemans:** Writing – review & editing. **J.H. von der Thüsen:** Conceptualization, Methodology, Investigation, Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lungcan.2022.12.018.

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