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Prashanth Ashok Kumar, Maryam Karimi, Alina Basnet, Lesley K. Seymour, Robert Arthur Kratzke, et al.. Association of Molecular Profiles and Mutational Status With Distinct Histological Lung Adenocarcinoma Subtypes. An Analysis of the LACE-Bio Data. *Clinical Lung Cancer*, 2023, 24 (6), pp.528-540. 10.1016/j.clcc.2023.06.002 . hal-04229530

HAL Id: hal-04229530

<https://hal.science/hal-04229530>

Submitted on 9 Apr 2024

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Association of Molecular Profiles and Mutational Status with Distinct Histological Lung Adenocarcinoma Subtypes. An Analysis of the LACE-Bio Data.

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38 **Conflict of Interest/Disclosures**

39 Prashanth Ashok Kumar: No disclosures or conflict of interest

40 Maryam Karimi: No disclosures or conflict of interest

41 Alina Basnet: No disclosures or conflict of interest

42 Lesley Seymour: Stock or Other Ownership: AstraZeneca, Consulting or Advisory Role:
43 Boehringer Ingelheim, Hanmi, Research Funding: Pfizer (Inst), AstraZeneca (Inst), Innate Pharma
44 (Inst), Oncolytics (Inst), Merck (Inst), Senwha (Inst).

45 Bob Kratzke: No disclosures or conflict of interest

46 Elizabeth Brambilla: No disclosures or conflict of interest

47 Thierry Le-Chevalier: No disclosures or conflict of interest

48 Jean-Charles Soria: Employment: MedImmune, Stock or Other Ownership:
49 AstraZeneca/MedImmune, Honoraria: Roche, AstraZeneca, Sanofi, Servier.

50 Lesley Seymour: Stock or Other Ownership: AstraZeneca, Consulting or Advisory Role:
51 Boehringer Ingelheim, Hanmi, Research Funding: Pfizer (Inst), AstraZeneca (Inst), Innate Pharma
52 (Inst), Oncolytics (Inst), Merck (Inst), Senwha (Inst).

53 Ken André Olausson: No disclosures or conflict of interest

54 Siddhartha Devarakonda: No disclosures or conflict of interest

55 Ramaswamy Govindan: Honoraria: AbbVie, Genentech, Consulting or Advisory Role: Pfizer,
56 Celgene, AstraZeneca, AbbVie, Merck, Inivata, EMD Serono, Genentech, Bristol-Myers Squibb,
57 Nektar, Merck Serono, Adaptimmune, Phillips Gilmore Oncology, GlaxoSmithKline, Research
58 Funding: National Cancer Institute.

59 Ming-Sound Tsao: No disclosures or conflict of interest

60 Frances A Shepherd: Stock or Other Ownership: Eli Lilly, AstraZeneca, Honoraria: Eli Lilly,
61 AstraZeneca, Bristol-Myers Squibb, Roche, Merck Sharp & Dohme, Merck Serono, Boehringer
62 Ingelheim, Consulting or Advisory Role: Eli Lilly, AstraZeneca, Boehringer Ingelheim, Merck
63 Serono, Research Funding: Eli Lilly (Inst), Pfizer (Inst), Bristol-Myers Squibb (Inst),
64 AstraZeneca/MedImmune (Inst), Roche Canada (Inst), Merrimack (Inst)

65 Stefan Michiels: Consulting or Advisory Role: IDDI, Hexal, Johnson & Johnson, Gentical,
66 Mabxience, Roche, QuintilesIMS, Patents, Royalties, Other Intellectual Property: Patent pending
67 of a prognostic gene score in early breast cancer: WO2017EP66533.

68 Stephen Graziano: No disclosures or conflict of interest

69

70 **Funding**

This work was supported by the Upstate University Hospital Department of Medicine Grant and National Institutes of Health Grant No. CA165958.

Ethics approval

The study was approved by the French Data Protection Committee.

All human investigations were conducted after approval by a local Human Investigations Committee and in accord with an assurance filed with and approved by the Department of Health and Human Services, where appropriate. Data has been anonymized and no identifiable information was used.

MicroAbstract

There are no reliable markers that would predict benefits of chemotherapy after surgery in limited stage lung adenocarcinoma. We used data from a repository of pooled historical trials to answer this question. It was noted that if TMB is high in these patients, chemotherapy use may have worse outcomes, and immune checkpoint therapy should be considered instead.

Abstract

Background

Adjuvant chemotherapy (AC) is indicated for stage II and stage III lung adenocarcinomas (ADC). Using the LACE Bio II database, we analyzed the distribution of various mutations across the subtypes of ADCs and studied the prognostic and predictive roles of PD-L1, TMB and Tumor Infiltrating Lymphocytes (TILs).

Methods

Clinical and genomic data from the LACE Bio II data were extracted. Patients were divided into ADC subtypes, in which the grouping was done based on their known clinical behavior [Lepidic (LEP), Acinar/Papillary (ACI or PAP), Micropapillary/Solid (MIP or SOL), Mucinous (MUC) and Others]. Kaplan-Meier (KM) and log-rank test were used to compare survival based on PD-L1, TMB, TILs and combinations of TMB with PD-L1 and TILs. Adjusted Hazard Ratios (HR) were analyzed with Overall Survival (OS), Disease-Free Survival (DFS) and Lung Cancer-Specific Survival (LCSS) as endpoints.

Results

375 ADC patients were identified. MIP/SOL was the subtype most commonly positive for various biomarkers. PD-L1 Negative/high TMB was associated with better outcomes in terms of OS (HR=0.46 [0.23-0.89], p=0.021) and DFS (HR=0.52 [0.30-0.90], p=0.02), relative to PD-L1

Negative/low TMB. High TMB predicted worse outcome with AC use in terms of OS (ratio of hazard ratio rHR=2.75 [1.07-7.04], p=0.035). Marked TILs had better outcome with AC for DFS (rHR=0.22 [0.06-0.87], p=0.031 and LCSS (rHR=0.08 [0.01-0.66], p=0.019) respectively. There was also a beneficial effect of AC among patients with Marked TILs/low TMB in terms of DFS (rHR= 0.06 [0.01-0.53], p=0.011).

Conclusions

High TMB has a prognostic role in resectable lung ADC. The high TMB group had a poor outcome with AC, suggesting that this group may be better served with immune checkpoint therapy.

Clinical Practice Points

It is known that adjuvant chemotherapy (AC) for limited stage lung adenocarcinoma (ADC) decreased the risk of death by 5.4% at 5 years. Given the toxicity associated with chemotherapy use, especially in patients with significant comorbidities, it is important to identify biomarkers that can predict response to AC. We performed a comprehensive analysis of the LACE Bio II data to help answer this. It was noted that the Micropapillary/Solid (MIP/SOL) subtype of lung adenocarcinoma is commonly associated with genetic alterations, indicating that this subtype has a high chance of expressing biomarkers that can be targeted in clinical practice. High TMB was shown to be associated with better prognosis overall. This combined with the result that high TMB patients had a poor outcome with AC use, questions the clinical utility of chemotherapy in this scenario. It also invokes the question of whether immune checkpoint therapy alone would better serve these patients with high TMB.

Keywords: Non-Small Cell Lung Cancer (NSCLC), Lung Adjuvant Cisplatin Evaluation (LACE), PD-L1, Tumor Mutational Burden (TMB), Tumor Infiltrating Lymphocytes (TIL), Adjuvant Chemotherapy

1

Abbreviations

AC- Adjuvant Chemotherapy; ADC-Adenocarcinoma, LACE- Lung Adjuvant Cisplatin Evaluation, LEP-Lepidic, ACI-Acinar, PAP-Papillary, MIP-Micropapillary, SOL-Solid, MUC-Mucinous, KM-Kaplan-Meier, PD-L1- Programmed death-ligand, TMB- Tumor mutational burden, TILs-Tumor Infiltrating Lymphocytes, HR-Hazard Ratio, rHR-Ratio of hazard ratio, OS-Overall Survival, DFS- Disease-Free Survival, LCSS-Lung Cancer-Specific Survival, NSCLC-Non-Small Cell Lung Cancer, IMC- International Multidisciplinary Classification, IASLC- International Association for the Study of Lung Cancer, ATS-American Thoracic Society, NCCN- National Comprehensive Cancer Network, ANITA-Adjuvant Navelbine International Trialist Association.

Introduction

Through the last century, lung cancer has emerged as not only the most common cancer, but also the most common cause of cancer related mortality, accounting for nearly 25% of the annual cancer related fatalities¹. While Small Cell Lung Cancer comprising 14% of all lung cancers is more aggressive, Non-Small Cell Lung Cancer (NSCLC) is more common and accounts for nearly 85% of the disease^{2,3}. The International Multidisciplinary Classification (IMC) is a collaborative, multidisciplinary effort by the International Association for the Study of Lung Cancer (IASLC), the American Thoracic Society (ATS), and the European Respiratory Society to provide a robust classification of lung adenocarcinoma (ADC) along with identification of prognostic and therapeutic targets. The various subtypes of ADC developed by the IMC and is now incorporated into the current WHO classification⁴ (Supplement S1)⁵. Research over the past 2 decades has shown that adjuvant chemotherapy (AC) has benefits compared to surgery alone in treating Stage II and Stage IIIA NSCLC. The current guidelines of the National Comprehensive Cancer Network (NCCN) recommends the same, especially after achieving complete surgical resection⁶. Immune checkpoint therapy have made significant progress, with agents like atezolizumab now found to be effective in the first line setting of all NSCLC subtypes with high PD-L1⁷. While the question of whether adjuvant or neoadjuvant chemotherapy is preferred remains at large, studies like McEnlay *et al.*'s review and the Lung Adjuvant Cisplatin Evaluation (LACE) have demonstrated survival advantage with adjuvant chemotherapy^{8,9}. In LACE, a large number (n = 4584) of completely resected NSCLC patients were analyzed from the 5 largest cisplatin-based adjuvant chemotherapy trials done after the 1995 NSCLC meta-analysis⁸. The LACE bio consortium involves investigators from the International Adjuvant Lung Trial, Cancer and Leukemia Group B-9633, National Cancer Institute of Canada Clinical Trials Group JBR.10, and Adjuvant Navelbine International Trialist Association (ANITA) trials¹⁰. LACE Bio contains comprehensive molecular characterization of patient samples from the above trials, except the ANITA trial as the tissue from the trial was exhausted¹⁰.

PDL-1 expression is believed to be predictive for response to immunotherapy but is not established for chemotherapy. Tsao *et al.* showed that PD-L1 was neither prognostic nor predictive of adjuvant chemotherapy use. Tumor Mutational Burden (TMB) can be defined as the total number of gene mutations per coding area of the entire tumor genome¹¹. In NSCLC, PD-L1 and TMB are independent biomarkers and have not been correlated with each other. Significant response rate with immunotherapy in different cancer types with higher TMB (> 10 Mut/Mb) has been studied and established. PD-L1 and TMB may overlap for prognostic and predictive response to immune checkpoint therapy but a distinct association has not been established for these in combination, especially for systemic chemotherapy¹².

NSCLC management continues to reach new frontiers as evident by the ADAURA clinical trial showing promising results with adjuvant osimertinib in *EGFR* mutated disease¹³. We wanted to contribute to the same end through our analysis using the LACE-Bio II data.

Methods

Study Design/Patient characteristics

We used data from the LACE-Bio II consortium including patients from 3 randomized clinical trials (JBR.10, IALT (International Adjuvant Lung Cancer Trial) and CALGB-9633) for whom FFPE samples were available.

ADC patients were extracted from LACE-Bio II data. Five subgroups were considered for the ADC patients: Lepidic (LEP), Acinar/Papillary (ACI or PAP), Micropapillary/Solid (MIP or SOL), Mucinous (MUC) and Other (Table 1). Combining the Acinar and Papillary subgroups and Micropapillary and Solid subgroups was performed based on the similar results in terms of survival outcomes in the literature¹⁴⁻¹⁷. Clinical and genomic data were used to provide a descriptive analysis of lung ADC based on histologic subtypes, tumor characteristics, selected targetable gene mutations and biomarkers. We also investigated the prognostic utility of the combinations of PDL-1, TMB and Tumor Infiltrating Lymphocytes (TILs) and if they can be used to predict benefit from adjuvant chemotherapy.

Biomarkers, genes, and copy number aberrations

We selected the following genes/biomarkers from LACE-Bio I: Mucin (0 vs. any positive staining), β -Tubulin (<180 vs. ≥ 180), p27 (<50 vs. ≥ 50), TILs (Marked vs. Non-marked/other), p53 (≤ 100 vs. >100), *KRAS* (Wilde type (WT) vs. Mutated (MUT)), *EGFR* (Wild type (WT) vs. Mutated (MUT)), ERCC1 (≤ 1 vs. >1), Cyclin E (<40 vs. ≥ 40), p16 (<1 vs. ≥ 1), FAS (<240 vs. ≥ 240), FASL (<240 vs. ≥ 240), BAX (<20 vs. ≥ 20), BRCA1 (<160 vs. ≥ 160), PD-L1 (negative: $<1\%$ vs. positive: $\geq 1\%$) and TMB (continuous and ≤ 10 vs. >10 Mut/Mb). More details on the techniques and thresholds used for these biomarkers can be found in previous LACE-Bio papers and has been summarized in the supplement¹⁸.

We also used a set of targetable genes from the LACE-Bio II TMB paper (1,538 genes selected based on the Cancer Genome Atlas Pan-Cancer analysis) to compare their frequencies between the 4 partly combined ADC subtype groups: lepidic, acinar/papillary, micropapillary/solid, mucinous (Others were not considered). Copy number aberrations were also considered to be compared across the 4 ADC subgroups (supplement)^{10,18-23}.

Outcomes

Following endpoints were considered as in previous publications from the consortium¹⁰:

- Overall Survival (OS), defined as the time from randomization to death from any cause,
- Disease-Free Survival (DFS), defined as the time from randomization to first recurrence (locoregional or distant) or death from any cause,
- Lung Cancer-Specific Survival (LCSS), defined as the time from randomization to death because of lung cancer.

207

208 **Statistical analysis**

209 The distribution of each biomarker was compared across 4 ADC subgroups (excluding ‘other’
210 subgroup) using Chi-squared test for binary biomarkers and Kruskal-Wallis test for continuous
211 biomarkers.

212 The frequency of mutated genes across the 4 ADC subgroups (excluding ‘other’) were compared
213 and all tests were corrected for multiple testing (FDR correction).

214 We investigated the association between Mucin expression status and *KRAS/EGFR* mutation,
215 association between PD-L1 expression and TMB/TILs, association between TILs and TMB. These
216 associations were assessed using separate logistic models with Mucin, PD-L1 expression and TILs
217 as the response variable. Likelihood ratio tests (LRT) were used to compare the models with and
218 without the biomarker of interest (*KRAS/EGFR*, TMB/TILs and TMB). All models were stratified
219 by trial and adjusted on treatment arm (unadjusted model), adjusted on treatment arm and clinical
220 covariates which included age, sex, tumor stage, nodal stage, WHO performance status, surgery
221 type (fully adjusted model) and adjusted on treatment arm, clinical covariates and ADC subtype
222 (sensitivity analysis). TMB was introduced in regressions as a continuous predictor. To relax the
223 assumption of linear relationship, the effect of TMB was also modeled in separate analyses using
224 spline with 3 knots.

225 The Kaplan-Meier (KM) method and log-rank test were used to compare survival curves between
226 the following groups:

- 227 - the two groups high TMB and low TMB,
- 228 - the two groups positive PD-L1 and low PD-L1,
- 229 - the four groups PD-L1 positive/high TMB, PD-L1 negative/high TMB, PD-L1
230 positive/low TMB, PD-L1 negative/low TMB,
- 231 - the two groups marked TILs and other,
- 232 - the four groups TILs marked/high TMB, TILs other/high TMB, TILs marked/low TMB,
233 TILs other/low TMB.

234 The biomarkers (PD-L1 expression, TMB, concomitant PD-L1 expression and TMB, TILs and
235 concomitant TMB and TILs) were correlated to each endpoint using a Cox proportional hazards
236 model, stratified by trial in all models, adjusted for treatment in the unadjusted model, adjusted for
237 treatment, age, sex, tumor stage, nodal stage, WHO performance status, surgery type in the fully
238 adjusted model, and adjusted for treatment, age, sex, tumor stage, nodal stage, WHO performance
239 status, surgery type and ADC subtype in the sensitivity analysis. In the analysis of PD-L1 and
240 TMB, the PD-L1 negative group, the low TMB group and the PD-L1 negative/low TMB group
241 were considered as the reference category. In the analysis of TILs and TMB, the TILs other group,
242 the low TMB group and the TILs other/low TMB group were considered as the reference category.

243 To evaluate the predictive role of PD-L1 expression, TMB status, concomitant PD-L1/TMB, TILs,
244 TMB status and concomitant TILs and TMB, a treatment-by-variable interaction was added to the
245 Cox models. We compared the treatment effect across groups of the variable of interest. For

example, for the concomitant PD-L1 expression and TMB status, we compared the treatment effect across four concomitant PD-L1 expression and TMB groups by using the ratio of HRs (rHR): the ratio of the HR for adjuvant chemotherapy versus observation in a given category (PD-L1 positive/High TMB, PD-L1 negative/High TMB, PD-L1 positive/low TMB) to the HR for adjuvant chemotherapy versus observation in the PD-L1 negative/low TMB category. Likewise, for the concomitant TILs and TMB status, we compared the treatment effect across four concomitant TILs and TMB groups by using the ratio of HRs (rHR): the ratio of the HR for adjuvant chemotherapy versus observation in each category (TILs Marked/High TMB, TILs other/High TMB, TILs Marked/low TMB) to the HR for adjuvant chemotherapy versus observation in the TILs Other/low TMB category. A rHR < 1.0 indicates that the treatment effect size was greater than that for PD-L1 negative/low TMB patients or TILs Other/low TMB patients. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using the ‘Survival’ and the ‘survminer’ packages in R software version 4.1.1.

Results

From the LACE-Bio II data, a total number of 375 ADC patients were identified. Out of these, 357 had correct inferred gender, 146 females and 211 males. Table 1 shows the characteristics of the study population.

The descriptive analysis of the biomarkers and genes from the LACE-Bio dataset has been represented in Table 2.A. Distribution of Mucin, β -Tubulin, p53, *KRAS*, Cyclin E and PD-L1 was significantly different across ADC subgroups. As anticipated, 95% of the MUC ADC stained positive for mucin. Among non-mucinous adenocarcinoma, ACI or PAP (65%) had the highest distribution of mucin positive patients. MIP/SOL was the subtype most commonly positive for biomarkers and genes, with significant difference seen in β -Tubulin (71%), p53(36%), Cyclin E (36%) and PD-L1 positivity (33%). *KRAS* mutation was seen most frequently in MUC (58%) followed by MIP/SOL (32%). Overall TMB ranged between 0.19 and 162.69 Mut/Mb. Median TMB was highest in MIP/SOL at 6.15 Mut/Mb and lowest in MUC at 2.24 Mut/Mb. MIP/SOL also had a higher likelihood of having high TMB by both levels as described in Table 2.A. Based on results from Table 2.B. no significant association between Mucin production and *KRAS/EGFR* status was observed.

Genetic analysis of the frequency of alterations in the LACE-Bio II data pool showed that *CNTNAP5*, *DRD5* and *EME1* were significantly differentially mutated between the 4 groups. *CNTNAP5* and *EME1* were frequently mutated in MIP/SOL, whereas *DRD5* was mutated commonly in LEP. More details on the frequencies and p-values can be found in the supplement. Copy number aberrations were investigated across ADC subgroups and analysis did not show any region with significantly different copy number aberration frequency between 4 ADC subgroups (Supplementary materials).

Supplement Table S2.A. shows the distribution of combinations of PD-L1 and TMB in the ADC subgroups from the LACE Bio II data, while the distribution of TILs and TMB is shown in Table

S2.B (supplement). Adjusting for clinico-pathological factors and ADC subtypes, higher TMB is associated with better DFS (HR = 0.65 [95%CI, 0.43-0.99], $p = 0.045$) by sensitivity analysis (Table 3.B). No significant prognostic effect was found for PD-L1 expression in terms of OS, DFS and LCSS. However, considering the concomitant PD-L1 expression and TMB status, analyses showed that concomitant PD-L1 negative/high TMB was associated with better outcomes in terms of OS (HR = 0.46 [95%CI, 0.23-0.89], $p = 0.021$) and DFS (HR = 0.53 [95%CI, 0.30-0.90], $p = 0.02$), relative to PD-L1 Negative/low TMB group. The results did not change adjusting for ADC subtypes (Table 3.A,B,C). The KM curves are shown in Figure S1 (supplement).

High TMB predicted worse outcome with adjuvant chemotherapy use in terms of OS (rHR = 2.75 [95%CI, 1.07-7.04], $p = 0.035$) (Table 4.A). Analyses (unadjusted, fully adjusted and sensitivity models) did not show any significant chemotherapy predictive effect of the PD-L1 expression and concomitant PD-L1 expression and TMB status in terms of OS, DFS and LCSS (Table 4.A,B,C). Unadjusted KM plots did not suggest any beneficial predictive effects for any of the biomarkers on OS, DFS and LCSS, when compared to observation (Supplement Figure S2.A,B,C).

On studying the prognostic effect of TMB and TILs, adjusted HR results suggested no significant prognostic effect for TILs in terms of OS, DFS and LCSS. However, considering the concomitant TILs and TMB status, sensitivity analyses alone showed that concomitant TILs other/high TMB was associated with better DFS (0.65 [95% CI, 0.43-0.97], $p = 0.037$) (Tables 5.A,B,C and supplement Figure S3).

Adjusted analyses (unadjusted, fully adjusted and sensitivity models) showed significant predictive effect of TILs, with beneficial effect of adjuvant chemotherapy among patients with marked TILs for DFS (rHR = 0.22 [95%CI, 0.06-0.87], $p = 0.031$) and LCSS (rHR = 0.08 [95%CI, 0.01-0.66], $p = 0.019$) respectively. In this cohort, it was once again seen from adjusted analyses that there was significant predictive effect of TMB in terms of OS with beneficial effect of adjuvant chemotherapy among patients with low TMB (rHR = 3.1 [95%CI, 1.25-7.7], $p = 0.015$). Also, there appears to be a beneficial effect of chemotherapy among patients with marked TILs/low TMB in terms of DFS (rHR = 0.06 [95%CI, 0.01-0.53], $p = 0.011$). (Table 6.A,B,C and Supplement Figure S4.A,B,C).

Discussion

The LACE-Bio project provides an opportunity to analyze a large number of lung ADC with robust clinical outcome data and detailed histopathological, immunohistochemical and molecular information²⁴. Our descriptive analysis shows that MIP/SOL (48%) was the commonest lung ADC subtype. There was a near 50-50 distribution of patients who received adjuvant chemotherapy and those who did not. Our cohort had a significant proportion of low-risk lung ADC as the majority were T2 (81%) and N0 (58%). Most of the patients underwent lobectomy (82%).

Analysis of prognostic and predictive factors, especially in relation to the pathological features of a patient's disease is an important part of clinical decision making. With data from the LACE-Bio I and II, we analyzed the association of lung ADC subtypes with potential and commonly used

genetic factors and biomarkers²². The lung ADC subtypes based on the current WHO Classification is shown in Table 1⁵. *KRAS* and *EGFR* mutations are mutually exclusive. Literature has shown that lepidic subtype is associated with *EGFR* mutation, whereas solid and mucinous subtypes are associated with lack of *EGFR*²⁵. Mucinous subtypes have shown strong association with *KRAS* expression, while lepidic and acinar are associated with lower prevalence of *KRAS* alterations²⁵. While our results reiterate the association of *KRAS* mutation with mucinous ADC, no association of *EGFR* with any of the ADC subtypes were observed. Although *KRAS* mutation is associated with mucinous subtypes, our results show that high mucin levels were not differentially associated with *KRAS* alternations or absence of *EGFR*.

MIP/SOL was the most common subtype that showed a significant association with biomarkers (β -Tubulin, p53, *KRAS*, Cyclin E, PD-L1 positivity, Median and high TMB). Several studies have revealed MIP and SOL histology to have a poor prognosis and worse outcomes in terms of recurrence free survival (RFS), disease free survival (DFS) and disease specific survival (DSS)^{26–28}. Reports have shown that MIP is more commonly associated with *EGFR* mutations than other subtypes. MIP patients with *EGFR* mutations also had better survival when treated with tyrosine kinase inhibitors or platinum based chemotherapy²⁹. Prospective analysis using NGS sequencing have provided evidence for MIP to have higher TMB, T cell infiltration and chromosomal instability³⁰. A prior analysis of Lace Bio studied the predictive value of ADC subtypes on benefit from adjuvant chemotherapy. DFS was poor with MIP/SOL compared to LEP, ACI or PAP. Survival advantage was observed with chemotherapy use in MIP/SOL patients (DFS: HR = 0.60 [95% CI, 0.44-0.82], $p = <0.01$, SDFS: HR = 0.59 [95% CI, 0.42-0.81], $p = 0.01$). Histologic subtyping may thus predict disease-specific outcomes with adjuvant chemotherapy use³¹. We can hypothesize that MIP/SOL, although more aggressive, has a higher chance of exhibiting molecular targets and may be more amenable to systemic therapy. Even though prognostic or predictive utility of biomarkers among ADC subtypes cannot be concluded, understanding their distribution enables a better understanding of these histological entities.

In the Checkmate 568 trial, TMB > 10 mut/Mb was associated with a better response and outcome when Stage IIIB and IV NSCLC was treated with nivolumab and low dose ipilimumab in both PD-L1 >1% and <1% subgroups³². TMB was traditionally measured by whole genome sequencing. In contemporary practice, targeted NGS like FoundationOne CDx is commonly used³³. Higher TMB levels are associated with MIP, ACI or SOL subtypes³⁴. This was consistent with our analysis. There was a near 50-50 distribution of TMB between high and low patients, among which MIP/SOL (59%) and ACI/PAP (47%) had the most patients with high TMB. Data from studies including past Lace Bio analysis indicate that the survival advantage of adjuvant chemotherapy is limited to tumors with N1 and N2 lymph node involvement. For N0 tumors, the benefit, albeit with a lower level of evidence, is limited to tumors greater than 4 cm in diameter. This is Stage IB in 7th edition and IIA in the 8th edition. Given significant toxicity with chemotherapy, besides the above-mentioned criteria, patient selection is based on comorbidities, recovery from surgery and functional status [8]. Despite the above evidence, the advantage of adjuvant chemotherapy for selected Stage IB, II and III NSCLC patients remains modest with a 5-year survival benefit of 5% and hazard ratio of 0.89 [6,8]. Currently, there are no validated molecular tools to aid patient selection for adjuvant chemotherapy [9–12]. Targeted sequencing of

908 samples involving 1538 genes selected based on the Cancer Genome Atlas (TCGA) Pan-Cancer analysis were reported in a previous LACE Bio publication. Devarakonda *et al.* using the above showed that a high nonsynonymous TMB (>8 mut/Mb) was associated with a better prognosis. The benefit of adjuvant chemotherapy in terms of LCSS was more pronounced with low nonsynonymous TMBs (<4 mut/Mb). The survival benefit, however, may be due to the small size and was not significant when the samples were regrouped by tertiles. The effect was noted in the overall subgroup of the study which included both ADC and SCC. The survival benefit was not observed when ADC alone was considered. This study used a TMB cut off as follows: low with ≤ 4 mut/Mb, intermediate with > 4 and ≤ 8 mut/M and high with > 8 mutations/Mb, while we used ≤ 10 vs. >10 which is more in line with current clinical practice¹⁰. Our cohort also included ADC alone and showed that low TMB predicted for better survival with adjuvant chemotherapy use for lung ADC. While PD-L1 alone and combinations of TMB and PD-L1 did not have any predictive effect, TILs however did have some significance in terms of DFS and LCSS.

The PACIFIC trial established the benefit of durvalumab in Stage III NSCLC. Following definitive chemoradiation, durvalumab maintenance for 12 months had a significantly better PFS (44.2 vs 27%), median duration of response (72.8 vs 46.8%) at 18 months, median time to death and metastasis (23.2 months vs. 14.6 months; $p<0.001$) and OS (0.68[99.73% CI, 0.47 -0.997], $p = 0.0025$)^{35,36}. While the KEYNOTE-189 established the superior OS and PFS of single agent pembrolizumab with standard chemotherapy in the untreated metastatic setting³⁷, the IMpower010 showed the benefit of adjuvant atezolizumab in Stage II-IIIa disease. Atezolizumab maintenance following adjuvant chemotherapy had better DFS (0.79 [95%CI, 0.64-0.96], $p = 0.0205$), with significant benefits in the PD-L1 Tumor cells $\geq 1\%$ subgroup (0.66 [95%CI, 0.50-0.88], $p = 0.0039$)³⁸. It is thus clear that TMB, PD-L1, and to a lesser extent TILs, are important factors in enabling providers to make a clinical decision. By providing information on the prognostic use and adjuvant chemotherapy predictive ability of the various combinations of these factors, a better understanding of the disease course may be possible. In our analysis, high TMB clearly had a survival advantage, establishing its role as a prognostic marker. The combination of PD-L1 negative/high TMB had better survival, but the exact clinical significance is uncertain and maybe because of the TMB being high. The high TMB group had a poor outcome with adjuvant chemotherapy use suggesting that this group may be better served with immune checkpoint therapy, rather than cytotoxic chemotherapy. TMB and PD-L1 combinations did not show any significance in predicting benefits of adjuvant chemotherapy. TILs alone did not have a significant prognostic survival advantage. TILs other/high TMB had some prognostic benefit, which could again be the result of TMB being high. Marked TILs did have a significance in predicting chemotherapy benefits in terms of DFS and LCSS, while marked TILs/low TMB had DFS benefit. This is hypothesis generating and can be the focus of future studies. Cancers with high TILs levels have shown to have higher sensitivity to chemotherapy in the past^{39,40}. High TILs are considered a favorable prognostic marker for NSCLC⁴¹. TILs thus have the potential to serve as a surrogate marker for a better outcome with adjuvant chemotherapy use in resectable lung ADC.

PD-L1 for pembrolizumab in modern practice is measured in terms of Tumor Proportion Score (TPS) using IHC like Dako PD-L1 IHC 22C3 pharmDx⁴². PD-L1 IHC in the LACE Bio data was carried out on 4 μ m sections, using the E1L3N rabbit monoclonal antibody (Cell Signaling,

Danvers, MA) on BenchMark XT autostainer (Ventana Medical Systems, Tucson, AZ) ²¹, which may yield differing results compared to current clinical assays ⁴³. This is the likely reason for a difference in percentage of PD-L1 positive patients seen in our analysis compared to contemporary clinical trials³⁷. This could be considered a limitation of our study. For several of the subtypes like LEP and MUC, the sample size was limited. Since the trials were done during the pre-immunotherapy era, the treatment selection was not prespecified and PD-L1, TMB and TILs analysis were done at a later point¹¹.

Our results reestablish and emphasize the application of the IASLC/ATS/ERS subtype classification and highlights their assessment in the context of presence or absence of prognostic and predictive biomarkers. MIP/SOL pattern is commonly associated with genetic alterations and shows positivity for several biomarkers. While high TMB is associated with better prognosis overall, its role in predicting survival with adjuvant chemotherapy is not established in lung ADC. The survival benefit seen with combinations noted above may be attributed to the presence of TMB being high in them. Marked TILs, although a small subgroup, may predict adjuvant chemotherapy response ⁴⁴. Patients with high TMB, given the poor outcome with adjuvant chemotherapy use, may be better served with immune checkpoint therapy. Further phase 3 studies are needed to establish this.

Acknowledgements

Our sincere thanks to the Upstate Cancer Center, the Upstate Department of Medicine grant, and the LACE Bio consortium for enabling us to carry out this analysis.

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Tables

Table 1. Demographic, clinical, and pathological characteristics of the study population.

Lace-Bio II	(N = 357) (%)
Age	
< 55	119 (33)
55-64	131 (37)
>= 65	107 (30)
Gender	
Female	146 (41)
Male	211 (59)
ADC subtype	
Lepidic (LEP)	14 (4)
Acinar/Papillary (ACI or PAP)	139 (39)
Micropapillary/Solid (MIP or SOL)	173 (48)
Mucinous (MUC)	19 (5)
Other	12 (3)
WHO performance status	
0	196 (55)
1-2	159 (45)
missing	2 (1)
T	
T1	52 (15)

T2	286 (81)
T3/T4	16 (5)
missing	3 (1)
N	
N0	206 (58)
N1	106 (30)
N2	42 (12)
missing	3 (1)
Stage	
IA	20 (6)
IB	181 (51)
IIA	25 (7)
IIB	81 (23)
IIIA	45 (13)
IIIB	2 (1)
missing	3 (1)
Surgery type	
Lobectomy/Other	294 (82)
Pneumonectomy	63 (18)
Treatment arm	
Observation	179 (50)
Adjuvant chemotherapy	178 (50)
Trial	
CALGB	74 (21)
IALT	163 (46)
JBR10	120 (34)

Table 2. A. Distribution of various biomarkers and genes from the Lace-Bio data in the overall population and subgroups according to ADC histologies. B. Association between Mucin and KRAS/EGFR.

A	Overall (N = 357) (%)	Lepidic (N = 14) (%)	Acinar or Papillary (N = 139) (%)	Micropapillary or Solid (N = 173) (%)	Mucinous (N = 19) (%)	Other (N = 12) (%)	P value (except other)
Mucin							4e-04^
0	116 (32)	6 (43)	39 (28)	69 (40)	0 (0)	2 (17)	
1 (any positive staining)	207 (58)	4 (29)	90 (65)	90 (52)	18 (95)	5 (42)	
Non evaluable	34 (10)	4 (29)	10 (7)	14 (8)	1 (5)	5 (42)	

β-Tubulin							5e-06^
0 (< 180)	115 (32)	7 (50)	57 (41)	36 (21)	12 (63)	3 (25)	
1 (>= 180)	208 (58)	6 (43)	70 (50)	123 (71)	5 (26)	4 (33)	
Non evaluable	34 (10)	1 (7)	12 (9)	14 (8)	2 (11)	5 (42)	
p27							0.6^
0 (< 50)	155 (43)	4 (29)	62 (45)	76 (44)	9 (47)	4 (33)	
1 (>= 50)	172 (48)	9 (64)	66 (47)	86 (50)	8 (42)	3 (25)	
Non evaluable	30 (8)	1 (7)	11 (8)	11 (6)	2 (11)	5 (42)	
TILs							0.7^
Marked	26 (7)	0 (0)	11 (8)	13 (8)	1 (5)	1 (8)	
Other	326 (91)	14 (100)	127 (91)	159 (92)	18 (95)	8 (67)	
Non evaluable	5 (1)	0 (0)	1 (1)	1 (1)	0 (0)	3 (25)	
p53							0.002^
0 (<= 100)	199 (56)	7 (50)	82 (59)	88 (51)	16 (84)	6 (50)	
1 (> 100)	97 (27)	1 (7)	34 (24)	62 (36)	0 (0)	0 (0)	
Non evaluable	61 (17)	6 (43)	23 (17)	23 (13)	3 (16)	6 (50)	
KRAS							0.04^
WT	238 (67)	9 (64)	98 (71)	116 (67)	7 (37)	8 (67)	
MUT	110 (31)	5 (36)	38 (27)	55 (32)	11 (58)	1 (8)	
Non evaluable	9 (3)	0 (0)	3 (2)	2 (1)	1 (5)	3 (25)	
EGFR							0.06^
WT	240 (67)	11 (79)	90 (65)	121 (70)	14 (74)	4 (33)	
MUT	36 (10)	2 (14)	21 (15)	12 (7)	0 (0)	1 (8)	
Non evaluable	81 (23)	1 (7)	28 (20)	40 (23)	5 (26)	7 (58)	
ERCC1							0.6^
0 (<=1)	13 (9)	0 (0)	7 (13)	6 (8)	0 (0)	0 (0)	
1 (> 1)	133 (91)	2 (100)	47 (87)	73 (92)	7 (100)	4 (100)	
Non evaluable	211 (59)	12 (86)	85 (61)	94 (54)	12 (63)	8 (67)	

Cyclin E							0.003 [^]
0 (< 40)	186 (73)	7 (100)	81 (82)	83 (64)	11 (92)	4 (67)	
1 (>= 40)	68 (27)	0 (0)	18 (18)	47 (36)	1 (8)	2 (33)	
Non evaluable	103 (29)	7 (50)	40 (29)	43 (25)	7 (37)	6 (50)	
p16							0.2 [^]
0 (< 1)	97 (38)	1 (14)	32 (32)	57 (44)	5 (42)	2 (33)	
1 (>= 1)	157 (62)	6 (86)	67 (68)	73 (56)	7 (58)	4 (67)	
Non evaluable	103 (29)	7 (50)	40 (29)	43 (25)	7 (37)	6 (50)	
FAS							0.4 [^]
0 (< 240)	252 (77)	9 (82)	104 (81)	124 (78)	12 (63)	3 (43)	
1 (>= 240)	74 (23)	2 (18)	25 (19)	36 (22)	7 (37)	4 (57)	
Non evaluable	31 (9)	3 (21)	10 (7)	13 (8)	0 (0)	5 (42)	
FASL							0.4 [^]
0 (< 240)	167 (51)	3 (27)	69 (53)	83 (52)	9 (47)	3 (43)	
1 (>= 240)	161 (49)	8 (73)	61 (47)	78 (48)	10 (53)	4 (57)	
Non evaluable	29 (8)	3 (21)	9 (6)	12 (7)	0 (0)	5 (42)	
BAX							0.1 [^]
0 (< 20)	62 (19)	1 (8)	17 (13)	37 (23)	5 (26)	2 (29)	
1 (>= 20)	263 (81)	11 (92)	109 (87)	124 (77)	14 (74)	5 (71)	
Non evaluable	32 (9)	2 (14)	13 (9)	12 (7)	0 (0)	5 (42)	
BRCA1							0.2 [^]
0 (< 160)	101 (44)	0 (0)	42 (45)	51 (44)	6 (46)	2 (40)	
1 (>= 160)	131 (56)	6 (100)	51 (55)	64 (56)	7 (54)	3 (60)	
Non evaluable	125 (35)	8 (57)	46 (33)	58 (34)	6 (32)	7 (58)	
PD-L1 (Tumor cell PD-L1 expression)							0.03 [^]
Negative (< 1%)	235 (73)	13 (100)	98 (77)	103 (67)	12 (75)	9 (90)	

Positive (>= 1%)	85 (27)	0 (0)	29 (23)	51 (33)	4 (25)	1 (10)			
Non evaluable	37 (10)	1 (7)	12 (9)	19 (11)	3 (16)	2 (17)			
TMB							4.16e-05*		
min	0.19	1.43	0.19	0.71	0.51	1.07			
median	4.87	2.43	4.33	6.15	2.24	3.23			
max	162.69	10.69	162.69	139.39	36.09	11.38			
mean (sd)	8.36 ± 14.64	3.77 ± 2.67	7.56 ± 15.03	9.98 ± 15.66	5.67 ± 9.43	3.88 ± 2.77			
TMB (3 levels as other papers)							0.0013^		
Low (<= 4 Mb)	147 (41)	9 (64)	63 (45)	54 (31)	14 (74)	7 (58)			
Moderate (4 Mb < <=7.8 Mb)	97 (27)	4 (29)	37 (27)	50 (29)	2 (11)	4 (33)			
High (> 7.8 Mb)	113 (32)	1 (7)	39 (28)	69 (40)	3 (16)	1 (8)			
TMB (2 levels by median)							0.0018^		
Low (<= 10 Mut/Mb)	279 (78)	13 (93)	114 (82)	125 (72)	16 (84)	11 (92)			
High (> 10 Mut/Mb)	78 (22)	1 (7)	25 (18)	48 (28)	3 (16)	1 (8)			
B	Unadjusted model		Fully adjusted model				Sensitivity analysis		
	OR [95% CI]	p	p(LRT)	OR [95% CI]	p	p(LRT)	OR [95% CI]	p	p(LRT)
KRAS (N = 312)									
MUT	1.03 [0.62-1.72]	0.909	0.909	1.05 [0.63-1.78]	0.85	0.85	0.96 [0.56-1.66]	0.897	0.897
WT	1.00			1.00			1.00		
EGFR (N = 243)									
MUT	1.13 [0.52-2.57]	0.766	0.765	1.05 [0.46-2.53]	0.902	0.902	1.07 [0.45-2.67]	0.878	0.878
WT	1.00			1.00			1.00		

592

593 ^ Chi-squared test

594 * Kruskal–Wallis test

Table 3. A. Prognostic effect of PD-L1 expression, TMB status and concomitant PD-L1 expression and TMB status on overall survival (OS) in the total population (n=315). B. Prognostic effect of PD-L1 expression, TMB status and concomitant PD-L1 expression and TMB status on disease-free survival (DFS) in the total population (n=315). C. Prognostic effect of PD-L1 expression, TMB status and concomitant PD-L1 expression and TMB status on lung-cancer-specific survival (LCSS) in the total population (n=315).

A.	Unadjusted model		Fully adjusted model		Sensitivity analysis	
	HR [95%CI]	p-value	HR [95%CI]	p-value	HR [95%CI]	p-value
PD-L1 expression						
PD-L1 Positive	1.33 [0.93-1.91]	0.116	1.15 [0.8-1.66]	0.455	1.16 [0.8-1.68]	0.437
PD-L1 Negative	1.00		1.00		1.00	
TMB Status						
TMB high	0.56 [0.36-0.88]	0.011	0.7 [0.44-1.11]	0.126	0.69 [0.43-1.11]	0.128
TMB low	1.00		1.00		1.00	
PD-L1/TMB						
PD-L1 Positive /high TMB	1.14 [0.63-2.05]	0.662	1.12 [0.62-2.05]	0.704	1.13 [0.61-2.07]	0.703
PD-L1 Positive /low TMB	1.11 [0.73-1.67]	0.625	0.98 [0.64-1.5]	0.929	0.98 [0.64-1.5]	0.932
PD-L1 Negative /high TMB	0.36 [0.19-0.69]	0.002	0.46 [0.23-0.89]	0.021	0.46 [0.23-0.89]	0.022
PD-L1 Negative /low TMB	1.00		1.00		1.00	
B.	Unadjusted model		Fully adjusted model		Sensitivity analysis	
	HR [95%CI]	p-value	HR [95%CI]	p-value	HR [95%CI]	p-value

PD-L1 expression						
PD-L1 Positive	1.39 [1-1.94]	0.053	1.28 [0.91-1.79]	0.16	1.28 [0.9-1.81]	0.164
PD-L1 Negative	1.00		1.00		1.00	
TMB Status						
TMB high	0.62 [0.42-0.91]	0.015	0.67 [0.44-1.01]	0.057	0.65 [0.43-0.99]	0.045
TMB low	1.00		1.00		1.00	
PD-L1/TMB						
PD-L1 Positive /high TMB	1.07 [0.62-1.85]	0.806	1.04 [0.6-1.82]	0.881	1.01 [0.58-1.78]	0.964
PD-L1 Positive /low TMB	1.27 [0.86-1.87]	0.223	1.2 [0.81-1.78]	0.372	1.19 [0.8-1.78]	0.388
PD-L1 Negative /high TMB	0.48 [0.29-0.81]	0.006	0.52 [0.3-0.9]	0.02	0.52 [0.3-0.9]	0.018
PD-L1 Negative /low TMB	1.00		1.00		1.00	
C.	Unadjusted model		Fully adjusted model		Sensitivity analysis	
	HR [95%CI]	p-value	HR [95%CI]	p-value	HR [95%CI]	p-value
PDL-L1 expression						
PD-L1 Positive	1.4 [0.98-2]	0.062	1.3 [0.9-1.87]	0.166	1.33 [0.91-1.93]	0.136
PD-L1 Negative	1.00		1.00		1.00	
TMB Status						

TMB high	0.63 [0.42-0.96]	0.03	0.71 [0.46-1.11]	0.137	0.71 [0.45-1.1]	0.126
TMB low	1.00		1.00		1.00	
PD-L1/TMB						
PD-L1 Positive /high TMB	1.01 [0.55-1.86]	0.963	1.03 [0.55-1.92]	0.926	1.03 [0.55-1.94]	0.915
PD-L1 Positive /low TMB	1.35 [0.9-2.04]	0.146	1.27 [0.84-1.93]	0.261	1.3 [0.85-1.98]	0.231
PD-L1 Negative/high TMB	0.54 [0.32-0.93]	0.026	0.61 [0.35-1.09]	0.094	0.62 [0.35-1.09]	0.094
PD-L1 Negative/low TMB	1.00		1.00		1.00	

Table 4.A. Predictive effect of PD-L1 expression, TMB status and concomitant PD-L1 expression and TMB status on overall survival (OS) in the total population (n=315). B. Predictive effect of PD-L1 expression, TMB status and concomitant PD-L1 expression and TMB status on disease-free survival (DFS) in the total population (n=315). C. Predictive effect of PD-L1 expression, TMB status and concomitant PD-L1 expression and TMB status on lung-cancer-specific survival (LCSS) in the total population (n=315)

A.	Unadjusted model		Fully adjusted model		Sensitivity analysis	
	rHR [95%CI]	p-value	rHR [95%CI]	p-value	rHR [95%CI]	p-value
PD-L1 expression						
PD-L1 Positive	1.05 [0.52-2.12]	0.9	1.08 [0.52-2.25]	0.827	1.07 [0.51-2.22]	0.86
PD-L1 Negative	1.00		1.00		1.00	

TMB Status						
TMB high	1.96 [0.79-4.84]	0.145	2.75 [1.07-7.04]	0.035	2.89 [1.13-7.4]	0.027
TMB low	1.00		1.00		1.00	
PD-L1/TMB						
PD-L1 Positive /high TMB	1.98 [0.56-6.93]	0.286	2.8 [0.78-10.03]	0.114	2.76 [0.77-9.9]	0.119
PD-L1 Positive /low TMB	0.87 [0.39-1.97]	0.742	0.77 [0.33-1.8]	0.553	0.78 [0.34-1.79]	0.552
PD-L1 Negative /high TMB	1.49 [0.42-5.27]	0.535	2.1 [0.56-7.85]	0.271	2.27 [0.6-8.52]	0.226
PD-L1 Negative /low TMB	1.00		1.00		1.00	
B.	Unadjusted model		Fully adjusted model		Sensitivity analysis	
	rHR [95%CI]	p-value	rHR [95%CI]	p-value	rHR [95%CI]	p-value
PDL-L1 expression						
PD-L1 Positive	1.1 [0.57-2.12]	0.774	1.06 [0.54-2.06]	0.869	1.05 [0.54-2.04]	0.895
PD-L1 Negative	1.00		1.00		1.00	
TMB Status						
TMB high	1.25 [0.58-2.71]	0.572	1.54 [0.69-3.4]	0.289	1.66 [0.75-3.65]	0.212
TMB low	1.00		1.00		1.00	
PD-L1/TMB						

PD-L1 Positive /high TMB	1.94 [0.62-6.1]	0.258	1.86 [0.58-5.96]	0.299	1.92 [0.6-6.16]	0.274
PD-L1 Positive /low TMB	0.81 [0.38-1.74]	0.593	0.82 [0.38-1.77]	0.607	0.81 [0.37-1.75]	0.588
PD-L1 Negative /high TMB	0.75 [0.26-2.13]	0.59	1.04 [0.35-3.09]	0.937	1.14 [0.39-3.35]	0.818
PD-L1 Negative /low TMB	1.00		1.00		1.00	
C.	Unadjusted model		Fully adjusted model		Sensitivity analysis	
	rHR [95%CI]	p-value	rHR [95%CI]	p-value	rHR [95%CI]	p-value
PDL-L1 expression						
PD-L1 Positive	1.02 [0.51-2.06]	0.954	0.96 [0.47-1.96]	0.908	0.95 [0.46-1.95]	0.895
PD-L1 Negative	1.00		1.00		1.00	
TMB Status						
TMB high	0.9 [0.39-2.06]	0.806	1.1 [0.47-2.57]	0.828	1.16 [0.5-2.72]	0.724
TMB low	1.00		1.00		1.00	
PD-L1/TMB						
PD-L1 Positive /high TMB	1.32 [0.39-4.51]	0.653	1.22 [0.35-4.24]	0.758	1.24 [0.36-4.34]	0.735
PD-L1 Positive /low TMB	0.82 [0.36-1.83]	0.621	0.83 [0.36-1.88]	0.651	0.83 [0.36-1.87]	0.646

PD-L1 Negative/high TMB	0.6 [0.2-1.8]	0.363	0.85 [0.27-2.68]	0.787	0.91 [0.29-2.85]	0.867
PD-L1 Negative/low TMB	1.00		1.00		1.00	

Table 5.A. Prognostic effect of TILs, TMB status and concomitant TILs and TMB status on overall survival (OS) in the total population (n=347). B. Prognostic effect of TILs, TMB status and concomitant TILs and TMB status on disease-free survival (DFS) in the total population (n=347). C. Prognostic effect of TILs, TMB status and concomitant TILs and TMB status on lung-cancer-specific survival (LCSS) in the total population (n=347).

A.	Unadjusted model		Fully adjusted model		Sensitivity analysis	
	HR [95%CI]	p-value	HR [95%CI]	p-value	HR [95%CI]	p-value
TILs						
Marked	0.6 [0.29-1.22]	0.159	0.54 [0.26-1.12]	0.099	0.54 [0.26-1.13]	0.102
Other	1.00		1.00		1.00	
TMB						
TMB high	0.57 [0.37-0.87]	0.009	0.72 [0.46-1.12]	0.146	0.71 [0.46-1.11]	0.138
TMB low	1.00		1.00		1.00	
TILs/TMB						
TILs Marked/high TMB	0.53 [0.13-2.14]	0.371	0.43 [0.1-1.79]	0.245	0.44 [0.1-1.84]	0.261
TILs Marked/low TMB	0.52 [0.23-1.18]	0.118	0.54 [0.23-1.27]	0.159	0.54 [0.23-1.27]	0.155
TILs Other/h	0.54 [0.35-0.84]	0.007	0.73 [0.46-1.16]	0.182	0.72 [0.46-1.15]	0.168

High TMB						
TILs Other/low TMB	1.00		1.00		1.00	
B.	Unadjusted model		Fully adjusted model		Sensitivity analysis	
	HR [95%CI]	p-value	HR [95%CI]	p-value	HR [95%CI]	p-value
TILs						
Marked	0.66 [0.36-1.22]	0.181	0.59 [0.31-1.12]	0.106	0.6 [0.32-1.13]	0.114
Other	1.00		1.00		1.00	
TMB Status						
TMB high	0.63 [0.43-0.91]	0.014	0.68 [0.46-1.01]	0.059	0.66 [0.44-0.99]	0.042
TMB low	1.00		1.00		1.00	
TILs/TMB						
TILs Marked/high TMB	0.65 [0.2-2.04]	0.457	0.54 [0.17-1.78]	0.312	0.55 [0.17-1.79]	0.32
TILs Marked/low TMB	0.56 [0.27-1.14]	0.11	0.54 [0.26-1.13]	0.103	0.54 [0.25-1.14]	0.106
TILs Other/high TMB	0.59 [0.4-0.88]	0.009	0.67 [0.44-1]	0.052	0.65 [0.43-0.97]	0.037
TILs Other/low TMB	1.00		1.00		1.00	
C.	Unadjusted model		Fully adjusted model		Sensitivity analysis	
	HR [95%CI]	p-value	HR [95%CI]	p-value	HR [95%CI]	p-value
TILs						

Marked	0.55 [0.27-1.12]	0.099	0.5 [0.24-1.04]	0.064	0.5 [0.24-1.06]	0.069
Other	1.00		1.00		1.00	
TMB Status						
TMB high	0.65 [0.44-0.97]	0.033	0.73 [0.48-1.11]	0.143	0.71 [0.46-1.09]	0.117
TMB low	1.00		1.00		1.00	
TILs/TMB						
TILs Marked /high TMB	0.51 [0.13-2.08]	0.35	0.46 [0.11-1.93]	0.289	0.48 [0.12-2.03]	0.321
TILs Marked /low TMB	0.48 [0.21-1.09]	0.081	0.46 [0.2-1.08]	0.076	0.46 [0.19-1.08]	0.074
TILs Other/high TMB	0.62 [0.42-0.94]	0.024	0.72 [0.46-1.11]	0.134	0.7 [0.45-1.08]	0.105
TILs Other/low TMB	1.00		1.00		1.00	

618

619 **Figure 6.A. Predictive effect of TILs, TMB status and concomitant TILs and TMB status on**
620 **overall survival (OS) in the total population (n=347). B. Predictive effect of TILs, TMB status**
621 **and concomitant TILs and TMB status on disease-free survival (DFS) in the total population**
622 **(n=347). C. Predictive effect of TILs, TMB status and concomitant TILs and TMB status on**
623 **lung-cancer-specific survival (LCSS) in the total population (n=347).**

A.	Unadjusted model		Fully adjusted model		Sensitivity analysis	
	rHR [95%CI]	p-value	rHR [95%CI]	p-value	rHR [95%CI]	p-value
TILs						
Marked	0.44 [0.1-1.92]	0.276	0.41 [0.09-1.84]	0.245	0.4 [0.09-1.81]	0.236
Other	1.00		1.00		1.00	

TMB						
TMB high	2.24 [0.93-5.4]	0.073	3.1 [1.25-7.7]	0.015	3.18 [1.28-7.88]	0.012
TMB low	1.00		1.00		1.00	
TILs/TMB						
TILs Marked /high TMB	0	0.994	0 [0-Inf]	0.995	0	0.992
TILs Marked /low TMB	0.13 [0.01-1.17]	0.069	0.12 [0.01-1.12]	0.063	0.12 [0.01-1.13]	0.064
TILs Other/high TMB	1.73 [0.7-4.25]	0.234	2.42 [0.96-6.1]	0.062	2.51 [1-6.32]	0.051
TILs Other/low TMB	1.00		1.00		1.00	
B.	Unadjusted model		Fully adjusted model		Sensitivity analysis	
	rHR [95%CI]	p-value	rHR [95%CI]	p-value	rHR [95%CI]	p-value
TILs						
Marked	0.24 [0.06-0.92]	0.038	0.22 [0.06-0.87]	0.031	0.23 [0.06-0.9]	0.035
Other	1.00		1.00		1.00	
TMB Status						
TMB high	1.52 [0.72-3.19]	0.273	1.87 [0.87-4]	0.107	1.95 [0.91-4.15]	0.085
TMB low	1.00		1.00		1.00	
TILs/TMB						
TILs Marked /high TMB	2.26 [0.2-25.71]	0.51	3.57 [0.3-43.11]	0.317	3.41 [0.28-40.76]	0.333

TILs Marked /low TMB	0.08 [0.01-0.64]	0.018	0.06 [0.01-0.53]	0.011	0.07 [0.01-0.57]	0.013
TILs Other/high TMB	1.24 [0.57-2.69]	0.584	1.47 [0.67-3.25]	0.337	1.56 [0.71-3.43]	0.267
TILs Other/low TMB	1.00		1.00		1.00	
C.	Unadjusted model		Fully adjusted model		Sensitivity analysis	
	rHR [95%CI]	p-value	rHR [95%CI]	p-value	rHR [95%CI]	p-value
TILs						
Marked	0.09 [0.01-0.71]	0.023	0.08 [0.01-0.66]	0.019	0.08 [0.01-0.66]	0.019
Other	1.00		1.00		1.00	
TMB Status						
TMB high	1.17 [0.53-2.57]	0.691	1.44 [0.64-3.23]	0.374	1.49 [0.67-3.33]	0.326
TMB low	1.00		1.00		1.00	
TILs/TMB						
TILs Marked /high TMB	0.99 [0.06-16.36]	0.997	1.41 [0.08-24.97]	0.813	1.35 [0.08-23.56]	0.836
TILs Marked /low TMB	0 [0-Inf]	0.992	0 [0-Inf]	0.993	0 [0-Inf]	0.993
TILs Other/high TMB	0.99 [0.44-2.23]	0.981	1.18 [0.51-2.71]	0.694	1.25 [0.55-2.85]	0.602
TILs Other/low TMB	1.00		1.00		1.00	

ow TMB						
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