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

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#### 74 **Ethics approval**

75 The study was approved by the French Data Protection Committee.

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

80

#### 81 **MicroAbstract**

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

86

#### 87 **Abstract**

##### 88 **Background**

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

##### 93 **Methods**

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

##### 101 **Results**

102 375 ADC patients were identified. MIP/SOL was the subtype most commonly positive for various  
103 biomarkers. PD-L1 Negative/high TMB was associated with better outcomes in terms of OS  
104 (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

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

## 110 **Conclusions**

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

113

## 114 **Clinical Practice Points**

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

126

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

130 <sup>1</sup>

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### 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-LI- 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.

131

## 132 **Introduction**

133 Through the last century, lung cancer has emerged as not only the most common cancer, but also  
134 the most common cause of cancer related mortality, accounting for nearly 25% of the annual cancer  
135 related fatalities <sup>1</sup>. While Small Cell Lung Cancer comprising 14% of all lung cancers is more  
136 aggressive, Non-Small Cell Lung Cancer (NSCLC) is more common and accounts for nearly 85%  
137 of the disease <sup>2,3</sup>. The International Multidisciplinary Classification (IMC) is a collaborative,  
138 multidisciplinary effort by the International Association for the Study of Lung Cancer (IASLC),  
139 the American Thoracic Society (ATS), and the European Respiratory Society to provide a robust  
140 classification of lung adenocarcinoma (ADC) along with identification of prognostic and  
141 therapeutic targets. The various subtypes of ADC developed by the IMC and is now incorporated  
142 into the current WHO classification<sup>4</sup> (Supplement S1)<sup>5</sup>. Research over the past 2 decades has  
143 shown that adjuvant chemotherapy (AC) has benefits compared to surgery alone in treating Stage  
144 II and Stage IIIA NSCLC. The current guidelines of the National Comprehensive Cancer Network  
145 (NCCN) recommends the same, especially after achieving complete surgical resection <sup>6</sup>. Immune  
146 checkpoint therapy have made significant progress, with agents like atezolizumab now found to  
147 be effective in the first line setting of all NSCLC subtypes with high PD-L1 <sup>7</sup>. While the question  
148 of whether adjuvant or neoadjuvant chemotherapy is preferred remains at large, studies like  
149 McEnlay *et al.*'s review and the Lung Adjuvant Cisplatin Evaluation (LACE) have demonstrated  
150 survival advantage with adjuvant chemotherapy <sup>8,9</sup>. In LACE, a large number (n = 4584) of  
151 completely resected NSCLC patients were analyzed from the 5 largest cisplatin-based adjuvant  
152 chemotherapy trials done after the 1995 NSCLC meta-analysis <sup>8</sup>. The LACE bio consortium  
153 involves investigators from the International Adjuvant Lung Trial, Cancer and Leukemia Group  
154 B-9633, National Cancer Institute of Canada Clinical Trials Group JBR.10, and Adjuvant  
155 Navelbine International Trialist Association (ANITA) trials <sup>10</sup>. LACE Bio contains comprehensive  
156 molecular characterization of patient samples from the above trials, except the ANITA trial as the  
157 tissue from the trial was exhausted <sup>10</sup>.

158 PDL-1 expression is believed to be predictive for response to immunotherapy but is not established  
159 for chemotherapy. Tsao *et al.* showed that PD-L1 was neither prognostic nor predictive of adjuvant  
160 chemotherapy use. Tumor Mutational Burden (TMB) can be defined as the total number of gene  
161 mutations per coding area of the entire tumor genome <sup>11</sup>. In NSCLC, PD-L1 and TMB are  
162 independent biomarkers and have not been correlated with each other. Significant response rate  
163 with immunotherapy in different cancer types with higher TMB (> 10 Mut/Mb) has been studied  
164 and established. PD-L1 and TMB may overlap for prognostic and predictive response to immune  
165 checkpoint therapy but a distinct association has not been established for these in combination,  
166 especially for systemic chemotherapy <sup>12</sup>.

167 NSCLC management continues to reach new frontiers as evident by the ADAURA clinical trial  
168 showing promising results with adjuvant osimertinib in *EGFR* mutated disease <sup>13</sup>. We wanted to  
169 contribute to the same end through our analysis using the LACE-Bio II data.

170

171

## 172 **Methods**

### 173 **Study Design/Patient characteristics**

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

177 ADC patients were extracted from LACE-Bio II data. Five subgroups were considered for the  
178 ADC patients: Lepidic (LEP), Acinar/Papillary (ACI or PAP), Micropapillary/Solid (MIP or  
179 SOL), Mucinous (MUC) and Other (Table 1). Combining the Acinar and Papillary subgroups and  
180 Micropapillary and Solid subgroups was performed based on the similar results in terms of survival  
181 outcomes in the literature<sup>14-17</sup>. Clinical and genomic data were used to provide a descriptive  
182 analysis of lung ADC based on histologic subtypes, tumor characteristics, selected targetable gene  
183 mutations and biomarkers. We also investigated the prognostic utility of the combinations of PDL-  
184 1, TMB and Tumor Infiltrating Lymphocytes (TILs) and if they can be used to predict benefit from  
185 adjuvant chemotherapy.

### 186 **Biomarkers, genes, and copy number aberrations**

187 We selected the following genes/biomarkers from LACE-Bio I: Mucin (0 vs. any positive  
188 staining),  $\beta$ -Tubulin (<180 vs.  $\geq$ 180), p27 (<50 vs.  $\geq$ 50), TILs (Marked vs. Non-marked/other),  
189 p53 ( $\leq$ 100 vs. >100), *KRAS* (Wild type (WT) vs. Mutated (MUT)), *EGFR* (Wild type (WT) vs.  
190 Mutated (MUT)), ERCC1 ( $\leq$ 1 vs. >1), Cyclin E (<40 vs.  $\geq$ 40), p16 (<1 vs.  $\geq$ 1), FAS (<240 vs.  
191  $\geq$ 240), FASL (<240 vs.  $\geq$ 240), BAX (<20 vs.  $\geq$ 20), BRCA1 (<160 vs.  $\geq$ 160), PD-L1 (negative:  
192 <1% vs. positive:  $\geq$ 1%) and TMB (continuous and  $\leq$ 10 vs. >10 Mut/Mb). More details on the  
193 techniques and thresholds used for these biomarkers can be found in previous LACE-Bio papers  
194 and has been summarized in the supplement<sup>18</sup>.

195 We also used a set of targetable genes from the LACE-Bio II TMB paper (1,538 genes selected  
196 based on the Cancer Genome Atlas Pan-Cancer analysis) to compare their frequencies between the  
197 4 partly combined ADC subtype groups: lepidic, acinar/papillary, micropapillary/solid, mucinous  
198 (Others were not considered). Copy number aberrations were also considered to be compared  
199 across the 4 ADC subgroups (supplement)<sup>10,18-23</sup>.

### 200 **Outcomes**

201 Following endpoints were considered as in previous publications from the consortium<sup>10</sup>:

- 202 - Overall Survival (OS), defined as the time from randomization to death from any cause,
- 203 - Disease-Free Survival (DFS), defined as the time from randomization to first recurrence  
204 (locoregional or distant) or death from any cause,
- 205 - Lung Cancer-Specific Survival (LCSS), defined as the time from randomization to death  
206 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



246 example, for the concomitant PD-L1 expression and TMB status, we compared the treatment effect  
247 across four concomitant PD-L1 expression and TMB groups by using the ratio of HRs (rHR): the  
248 ratio of the HR for adjuvant chemotherapy versus observation in a given category (PDL-1  
249 positive/High TMB, PD-L1 negative/High TMB, PD-L1 positive/low TMB) to the HR for  
250 adjuvant chemotherapy versus observation in the PD-L1 negative/low TMB category. Likewise,  
251 for the concomitant TILs and TMB status, we compared the treatment effect across four  
252 concomitant TILs and TMB groups by using the ratio of HRs (rHR): the ratio of the HR for  
253 adjuvant chemotherapy versus observation in each category (TILs Marked/High TMB, TILs  
254 other/High TMB, TILs Marked/low TMB) to the HR for adjuvant chemotherapy versus  
255 observation in the TILs Other/low TMB category. A rHR < 1.0 indicates that the treatment effect  
256 size was greater than that for PD-L1 negative/low TMB patients or TILs Other/low TMB patients.

257 Statistical significance was set at  $p < 0.05$ . All statistical analyses were performed using the  
258 'Survival' and the 'survminer' packages in R software version 4.1.1.

259

## 260 **Results**

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

264 The descriptive analysis of the biomarkers and genes from the LACE-Bio dataset has been  
265 represented in Table 2.A. Distribution of Mucin,  $\beta$ -Tubulin, p53, *KRAS*, Cyclin E and PD-L1 was  
266 significantly different across ADC subgroups. As anticipated, 95% of the MUC ADC stained  
267 positive for mucin. Among non-mucinous adenocarcinoma, ACI or PAP (65%) had the highest  
268 distribution of mucin positive patients. MIP/SOL was the subtype most commonly positive for  
269 biomarkers and genes, with significant difference seen in  $\beta$ -Tubulin (71%), p53(36%), Cyclin E  
270 (36%) and PD-L1 positivity (33%). *KRAS* mutation was seen most frequently in MUC (58%)  
271 followed by MIP/SOL (32%). Overall TMB ranged between 0.19 and 162.69 Mut/Mb. Median  
272 TMB was highest in MIP/SOL at 6.15 Mut/Mb and lowest in MUC at 2.24 Mut/Mb. MIP/SOL  
273 also had a higher likelihood of having high TMB by both levels as described in Table 2.A. Based  
274 on results from Table 2.B. no significant association between Mucin production and *KRAS/EGFR*  
275 status was observed.

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

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

285 S2.B (supplement). Adjusting for clinico-pathological factors and ADC subtypes, higher TMB is  
286 associated with better DFS (HR = 0.65 [95%CI, 0.43-0.99], p = 0.045) by sensitivity analysis  
287 (Table 3.B). No significant prognostic effect was found for PD-L1 expression in terms of OS, DFS  
288 and LCSS. However, considering the concomitant PD-L1 expression and TMB status, analyses  
289 showed that concomitant PD-L1 negative/high TMB was associated with better outcomes in terms  
290 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 =  
291 0.02), relative to PD-L1 Negative/low TMB group. The results did not change adjusting for ADC  
292 subtypes (Table 3.A,B,C). The KM curves are shown in Figure S1 (supplement).

293 High TMB predicted worse outcome with adjuvant chemotherapy use in terms of OS (rHR = 2.75  
294 [95%CI, 1.07-7.04], p = 0.035) (Table 4.A). Analyses (unadjusted, fully adjusted and sensitivity  
295 models) did not show any significant chemotherapy predictive effect of the PD-L1 expression and  
296 concomitant PD-L1 expression and TMB status in terms of OS, DFS and LCSS (Table 4.A,B,C).  
297 Unadjusted KM plots did not suggest any beneficial predictive effects for any of the biomarkers  
298 on OS, DFS and LCSS, when compared to observation (Supplement Figure S2.A,B,C).

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

304 Adjusted analyses (unadjusted, fully adjusted and sensitivity models) showed significant  
305 predictive effect of TILs, with beneficial effect of adjuvant chemotherapy among patients with  
306 marked TILs for DFS (rHR = 0.22 [95%CI, 0.06-0.87], p = 0.031) and LCSS (rHR = 0.08 [95%CI,  
307 0.01-0.66], p = 0.019) respectively. In this cohort, it was once again seen from adjusted analyses  
308 that there was significant predictive effect of TMB in terms of OS with beneficial effect of adjuvant  
309 chemotherapy among patients with low TMB (rHR = 3.1 [95%CI, 1.25-7.7], p = 0.015). Also,  
310 there appears to be a beneficial effect of chemotherapy among patients with marked TILs/low  
311 TMB in terms of DFS (rHR = 0.06 [95%CI, 0.01-0.53], p = 0.011). (Table 6.A,B,C and  
312 Supplement Figure S4.A,B,C).

313

## 314 **Discussion**

315 The LACE-Bio project provides an opportunity to analyze a large number of lung ADC with robust  
316 clinical outcome data and detailed histopathological, immunohistochemical and molecular  
317 information<sup>24</sup>. Our descriptive analysis shows that MIP/SOL (48%) was the commonest lung ADC  
318 subtype. There was a near 50-50 distribution of patients who received adjuvant chemotherapy and  
319 those who did not. Our cohort had a significant proportion of low-risk lung ADC as the majority  
320 were T2 (81%) and N0 (58%). Most of the patients underwent lobectomy (82%).

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

324 genetic factors and biomarkers <sup>22</sup>. The lung ADC subtypes based on the current WHO  
325 Classification is shown in Table 1<sup>5</sup>. *KRAS* and *EGFR* mutations are mutually exclusive. Literature  
326 has shown that lepidic subtype is associated with *EGFR* mutation, whereas solid and mucinous  
327 subtypes are associated with lack of *EGFR*<sup>25</sup>. Mucinous subtypes have shown strong association  
328 with *KRAS* expression, while lepidic and acinar are associated with lower prevalence of *KRAS*  
329 alterations <sup>25</sup>. While our results reiterate the association of *KRAS* mutation with mucinous ADC,  
330 no association of *EGFR* with any of the ADC subtypes were observed. Although *KRAS* mutation  
331 is associated with mucinous subtypes, our results show that high mucin levels were not  
332 differentially associated with *KRAS* alternations or absence of *EGFR*.

333 MIP/SOL was the most common subtype that showed a significant association with biomarkers  
334 ( $\beta$ -Tubulin, p53, *KRAS*, Cyclin E, PD-L1 positivity, Median and high TMB). Several studies have  
335 revealed MIP and SOL histology to have a poor prognosis and worse outcomes in terms of  
336 recurrence free survival (RFS), disease free survival (DFS) and disease specific survival (DSS) <sup>26-</sup>  
337 <sup>28</sup>. Reports have shown that MIP is more commonly associated with *EGFR* mutations than other  
338 subtypes. MIP patients with *EGFR* mutations also had better survival when treated with tyrosine  
339 kinase inhibitors or platinum based chemotherapy <sup>29</sup>. Prospective analysis using NGS sequencing  
340 have provided evidence for MIP to have higher TMB, T cell infiltration and chromosomal  
341 instability <sup>30</sup>. A prior analysis of Lace Bio studied the predictive value of ADC subtypes on benefit  
342 from adjuvant chemotherapy. DFS was poor with MIP/SOL compared to LEP, ACI or PAP.  
343 Survival advantage was observed with chemotherapy use in MIP/SOL patients (DFS: HR = 0.60  
344 [95% CI, 0.44-0.82], p = <0.01, SDFS: HR = 0.59 [95% CI, 0.42-0.81], p = 0.01). Histologic  
345 subtyping may thus predict disease-specific outcomes with adjuvant chemotherapy use <sup>31</sup>. We can  
346 hypothesize that MIP/SOL, although more aggressive, has a higher chance of exhibiting molecular  
347 targets and may be more amenable to systemic therapy. Even though prognostic or predictive  
348 utility of biomarkers among ADC subtypes cannot be concluded, understanding their distribution  
349 enables a better understanding of these histological entities.

350 In the Checkmate 568 trial, TMB > 10 mut/Mb was associated with a better response and outcome  
351 when Stage IIIB and IV NSCLC was treated with nivolumab and low dose ipilimumab in both  
352 PD-L1 >1% and <1% subgroups <sup>32</sup>. TMB was traditionally measured by whole genome  
353 sequencing. In contemporary practice, targeted NGS like FoundationOne CDx is commonly used  
354 <sup>33</sup>. Higher TMB levels are associated with MIP, ACI or SOL subtypes <sup>34</sup>. This was consistent with  
355 our analysis. There was a near 50-50 distribution of TMB between high and low patients, among  
356 which MIP/SOL (59%) and ACI/PAP (47%) had the most patients with high TMB. Data from  
357 studies including past Lace Bio analysis indicate that the survival advantage of adjuvant  
358 chemotherapy is limited to tumors with N1 and N2 lymph node involvement. For N0 tumors, the  
359 benefit, albeit with a lower level of evidence, is limited to tumors greater than 4 cm in diameter.  
360 This is Stage IB in 7th edition and IIA in the 8th edition. Given significant toxicity with  
361 chemotherapy, besides the above-mentioned criteria, patient selection is based on comorbidities,  
362 recovery from surgery and functional status [8]. Despite the above evidence, the advantage of  
363 adjuvant chemotherapy for selected Stage IB, II and III NSCLC patients remains modest with a 5-  
364 year survival benefit of 5% and hazard ratio of 0.89 [6,8]. Currently, there are no validated  
365 molecular tools to aid patient selection for adjuvant chemotherapy [9–12]. Targeted sequencing of

366 908 samples involving 1538 genes selected based on the Cancer Genome Atlas (TCGA) Pan-  
367 Cancer analysis were reported in a previous LACE Bio publication. Devarakonda *et al.* using the  
368 above showed that a high nonsynonymous TMB (>8 mut/Mb) was associated with a better  
369 prognosis. The benefit of adjuvant chemotherapy in terms of LCSS was more pronounced with  
370 low nonsynonymous TMBs (<4 mut/Mb). The survival benefit, however, may be due to the small  
371 size and was not significant when the samples were regrouped by tertiles. The effect was noted in  
372 the overall subgroup of the study which included both ADC and SCC. The survival benefit was  
373 not observed when ADC alone was considered. This study used a TMB cut off as follows: low  
374 with  $\leq 4$  mut/Mb, intermediate with  $> 4$  and  $\leq 8$  mut/M and high with  $> 8$  mutations/Mb, while  
375 we used  $\leq 10$  vs.  $> 10$  which is more in line with current clinical practice<sup>10</sup>. Our cohort also included  
376 ADC alone and showed that low TMB predicted for better survival with adjuvant chemotherapy  
377 use for lung ADC. While PD-L1 alone and combinations of TMB and PD-L1 did not have any  
378 predictive effect, TILs however did have some significance in terms of DFS and LCSS.

379 The PACIFIC trial established the benefit of durvalumab in Stage III NSCLC. Following definitive  
380 chemoradiation, durvalumab maintenance for 12 months had a significantly better PFS (44.2 vs  
381 27%), median duration of response (72.8 vs 46.8%) at 18 months, median time to death and  
382 metastasis (23.2 months vs. 14.6 months;  $p < 0.001$ ) and OS (0.68 [99.73% CI, 0.47 -0.997],  $p =$   
383 0.0025)<sup>35,36</sup>. While the KEYNOTE-189 established the superior OS and PFS of single agent  
384 pembrolizumab with standard chemotherapy in the untreated metastatic setting<sup>37</sup>, the IMpower010  
385 showed the benefit of adjuvant atezolizumab in Stage II-IIIa disease. Atezolizumab maintenance  
386 following adjuvant chemotherapy had better DFS (0.79 [95%CI, 0.64-0.96],  $p = 0.0205$ ), with  
387 significant benefits in the PD-L1 Tumor cells  $\geq 1\%$  subgroup (0.66 [95%CI, 0.50-0.88],  $p = 0.0039$ )  
388<sup>38</sup>. It is thus clear that TMB, PD-L1, and to a lesser extent TILs, are important factors in enabling  
389 providers to make a clinical decision. By providing information on the prognostic use and adjuvant  
390 chemotherapy predictive ability of the various combinations of these factors, a better  
391 understanding of the disease course may be possible. In our analysis, high TMB clearly had a  
392 survival advantage, establishing its role as a prognostic marker. The combination of PD-L1  
393 negative/high TMB had better survival, but the exact clinical significance is uncertain and maybe  
394 because of the TMB being high. The high TMB group had a poor outcome with adjuvant  
395 chemotherapy use suggesting that this group may be better served with immune checkpoint  
396 therapy, rather than cytotoxic chemotherapy. TMB and PD-L1 combinations did not show any  
397 significance in predicting benefits of adjuvant chemotherapy. TILs alone did not have a significant  
398 prognostic survival advantage. TILs other/high TMB had some prognostic benefit, which could  
399 again be the result of TMB being high. Marked TILs did have a significance in predicting  
400 chemotherapy benefits in terms of DFS and LCSS, while marked TILs/low TMB had DFS benefit.  
401 This is hypothesis generating and can be the focus of future studies. Cancers with high TILs levels  
402 have shown to have higher sensitivity to chemotherapy in the past<sup>39,40</sup>. High TILs are considered  
403 a favorable prognostic marker for NSCLC<sup>41</sup>. TILs thus have the potential to serve as a surrogate  
404 marker for a better outcome with adjuvant chemotherapy use in resectable lung ADC.

405 PD-L1 for pembrolizumab in modern practice is measured in terms of Tumor Proportion Score  
406 (TPS) using IHC like Dako PD-L1 IHC 22C3 pharmDx<sup>42</sup>. PD-L1 IHC in the LACE Bio data was  
407 carried out on 4  $\mu$ m sections, using the E1L3N rabbit monoclonal antibody (Cell Signaling,

408 Danvers, MA) on BenchMark XT autostainer (Ventana Medical Systems, Tucson, AZ) <sup>21</sup>, which  
409 may yield differing results compared to current clinical assays <sup>43</sup>. This is the likely reason for a  
410 difference in percentage of PD-L1 positive patients seen in our analysis compared to contemporary  
411 clinical trials<sup>37</sup>. This could be considered a limitation of our study. For several of the subtypes like  
412 LEP and MUC, the sample size was limited. Since the trials were done during the pre-  
413 immunotherapy era, the treatment selection was not prespecified and PD-L1, TMB and TILs  
414 analysis were done at a later point<sup>11</sup>.

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

425

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429

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581

582

583 **Tables**

584

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

586

<b>Lace-Bio II</b>	<b>(N = 357) (%)</b>
<b>Age</b>	
< 55	119 (33)
55-64	131 (37)
>= 65	107 (30)
<b>Gender</b>	
Female	146 (41)
Male	211 (59)
<b>ADC subtype</b>	
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)
<b>WHO performance status</b>	
0	196 (55)
1-2	159 (45)
missing	2 (1)
<b>T</b>	
T1	52 (15)

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

587

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

591

<b>A</b>	<b>Overall (N = 357) (%)</b>	<b>Lepidic (N = 14) (%)</b>	<b>Acinar or Papillary (N = 139) (%)</b>	<b>Micropapillary or Solid (N = 173) (%)</b>	<b>Mucinous (N = 19) (%)</b>	<b>Other (N = 12) (%)</b>	<b>P value (except other)</b>
<b>Mucin</b>							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)	

<b><math>\beta</math>-Tubulin</b>							5e-06 <sup>^</sup>
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)	
<b>p27</b>							0.6 <sup>^</sup>
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)	
<b>TILs</b>							0.7 <sup>^</sup>
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)	
<b>p53</b>							0.002 <sup>^</sup>
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)	
<b>KRAS</b>							0.04 <sup>^</sup>
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)	
<b>EGFR</b>							0.06 <sup>^</sup>
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)	
<b>ERCC1</b>							0.6 <sup>^</sup>
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)	

<b>Cyclin E</b>							0.003 <sup>^</sup>
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)	
<b>p16</b>							0.2 <sup>^</sup>
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)	
<b>FAS</b>							0.4 <sup>^</sup>
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)	
<b>FASL</b>							0.4 <sup>^</sup>
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)	
<b>BAX</b>							0.1 <sup>^</sup>
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)	
<b>BRCA1</b>							0.2 <sup>^</sup>
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)	
<b>PD-L1 (Tumor cell PD-L1 expression)</b>							0.03 <sup>^</sup>
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)			
<b>TMB</b>									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			
<b>TMB (3 levels as other papers)</b>									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)			
<b>TMB (2 levels by median)</b>									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>B</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)
<b>KRAS (N = 312)</b>									
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		
<b>EGFR (N = 243)</b>									
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

595

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

602

A.	Unadjusted model		Fully adjusted model		Sensitivity analysis	
	HR [95%CI]	p-value	HR [95%CI]	p-value	HR [95%CI]	p-value
<b>PD-L1 expression</b>						
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	
<b>TMB Status</b>						
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	
<b>PD-L1/TMB</b>						
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

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

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

603

604

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

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



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

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

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

611

612

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

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

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

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

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

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

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

<b>ow</b> <b>TMB</b>						
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