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## Impaired Tumor-Infiltrating T Cells in Patients with Chronic Obstructive Pulmonary Disease Impact Lung Cancer Response to PD-1 Blockade

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1 **Impaired tumor-infiltrating T cells in patients with COPD impacts lung cancer response to**

2 **PD-1 blockade**

3

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32

33 **Author contributions:** D.D and R.H designed and supervised the study. J.B, H.O, J.G, H.K,

34 P.D-M and C.G acquired immunohistochemical data. J.B acquired flow cytometry data. H.S,

35 Reb.H and T.C acquired and analyzed WES experiments. J.B, H.O, C.B-M, C.G, R.H and D.D

36 analyzed the data. J.B and A.D performed statistical analysis. H.O, A.M-L, M-A and D-D were

37 responsible for clinical data. A.M-L and D.D were responsible for pathological data. J.B, R.H and

38 D.D interpreted data. J.B, D.D and R.H wrote the manuscript. M.A, H.O, N.R, P-R.B, C.G, I.C

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40

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45

46 **Running title:** COPD disrupts NSCLC tumor immune contexture.

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48 diseases/9.09 COPD: General; Immunology and inflammation/7.15 Lymphocytes.

49 **Total word count for the body of the manuscript:** 4689 words.

50 **At the Glance Commentary:**

51 Scientific knowledge on the subject: The immune system is strongly involved in the  
52 establishment of chronic inflammation in chronic obstructive pulmonary disease (COPD) and in  
53 the control of tumor burden in lung cancer. However, despite the strong epidemiological link  
54 between these two diseases, the impact of COPD associated chronic inflammation on the immune  
55 contexture of lung cancer remains poorly defined.

56 What this study adds to the field: Here, we report that COPD disrupts the immune  
57 microenvironment of non-small cell lung cancer (NSCLC), and we identify CD8 tumor  
58 infiltrating lymphocytes (CD8 TILs) as the most affected population. Indeed, we observed higher  
59 exhaustion of CD8 TILs, identified by PD-1/TIM-3 coexpression, in NSCLC patients with  
60 coexisting moderate to severe COPD. In agreement, the prognostic value of intra-tumor CD8<sup>+</sup> T  
61 cells that has been found favorable in most cancer types and particularly in NSCLC, has no  
62 impact on the survival of patients with coexisting COPD. Together, our data point out COPD  
63 patients as a potential NSCLC patient population to treat with immune-checkpoint blockers. In  
64 accordance with this hypothesis, data obtained in a cohort of 39 nivolumab treated patients might  
65 suggest a higher efficacy of anti-PD-1 treatment in NSCLC patients with a coexisting COPD.

66

67 This article has an online data supplement, which is accessible from this issue's table of content  
68 online at [www.atsjournals.org](http://www.atsjournals.org).



69 **Abstract**

70 **Rationale:** Patients with chronic obstructive pulmonary disease (COPD) have a higher  
71 prevalence of lung cancer. The chronic inflammation associated with COPD probably promotes  
72 the earliest stages of carcinogenesis. However, once tumors have progressed to malignancy, the  
73 impact of COPD on the tumor immune microenvironment remains poorly defined, and its effects  
74 on immune-checkpoint blockers' efficacy are still unknown.

75 **Objectives:** To study the impact of COPD on the immune contexture of non-small cell lung  
76 cancer (NSCLC).

77 **Methods:** We performed in depth immune profiling of lung tumors by immunohistochemistry  
78 and we determined its impact on patients' survival (n=435). Tumor-infiltrating T lymphocyte  
79 (TILs) exhaustion by flow cytometry (n=50) was also investigated. The effectiveness of an anti-  
80 PD-1 treatment (nivolumab) was evaluated in 39 advanced-stage NSCLC patients. All data were  
81 analyzed according to patients' COPD status.

82 **Measurements and Main Results:** Remarkably, COPD severity is positively correlated with the  
83 coexpression of PD-1/TIM-3 by CD8 T cells. In agreement, we observed a loss of CD8 T cell-  
84 associated favorable clinical outcome in COPD<sup>+</sup> patients. Interestingly, a negative prognostic  
85 value of PD-L1 expression by tumor cells was observed only in highly CD8 T cell-infiltrated  
86 tumors of COPD<sup>+</sup> patients. Finally, data obtained on 39 advanced-stage NSCLC patients treated  
87 by an anti-PD-1 antibody showed longer progression free survival in COPD<sup>+</sup> patients, and also  
88 that the association between the severity of smoking and the response to nivolumab was  
89 preferentially observed in COPD<sup>+</sup> patients.

90 **Conclusions:** COPD is associated with an increased sensitivity of CD8 TILs to immune escape  
91 mechanisms developed by tumors, thus suggesting a higher sensitivity to PD-1 blockade in  
92 patients with COPD.

93 **Keywords:** NSCLC, tumor immunology, CD8 TILs, anti-PD-1.

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95 **Total word count for the abstract:** 250 words

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## 117 **Introduction**

118           Despite abundant evidence that the immune system plays a central role in controlling  
119 tumor burden (1–3), it may also have a dark side linked to the maintenance of deleterious  
120 inflammation. For instance, patients with inflammatory bowel disease (IBD) (4) or chronic  
121 pancreatitis (5) showed increased risk of developing colorectal and pancreatic cancer,  
122 respectively. Similarly, chronic obstructive pulmonary disease (COPD) is considered to be an  
123 important risk factor for lung cancer (6, 7). This inflammatory condition is linked to a more  
124 pronounced destructive inflammation of the lung, compared with non-COPD smokers,  
125 characterized by a strong release of TNF- $\alpha$  and CXCL-8 by epithelial cells and alveolar  
126 macrophages leading to the recruitment of inflammatory monocytes and neutrophils (8). The  
127 presence of B cells in lymphoid follicles has been reported in the airways and parenchyma of  
128 patients with COPD (9), illustrating the involvement of adaptive immunity in COPD  
129 pathophysiology. This chronic inflammation may promote the earliest stages of carcinogenesis  
130 (8) through an increased expression of genes involved in cell proliferation and survival, including  
131 NF- $\kappa$ B and STAT3, which are activated by cytokines such as IL-6 and TNF- $\alpha$ .

132           Once tumors have progressed to malignancy, COPD was shown to worsen the survival of  
133 patients with early-stage non-small cell lung cancer (NSCLC) (10) and emphysema was shown to  
134 be associated with increased lung cancer mortality (11). Mechanisms governing this prognostic  
135 impact, including the role of the immune system, are currently undefined. Although the tumor  
136 immune contexture in NSCLC has been extensively characterized, the COPD status of patients  
137 has not been taken into account. Nevertheless, a high density of CD8 tumor-infiltrating T  
138 lymphocytes (CD8 TILs), together with a concomitant high density of DC-Lamp<sup>+</sup> cells that  
139 signals the presence of tertiary lymphoid structures (TLS) within tumor tissues, identified patients  
140 with the best prognostic outcome (12). However, overexpression of inhibitory receptors by



141 tumor-infiltrating T cells, also called immune-checkpoints, can keep the immune system under  
142 control (13). In NSCLC, their cumulative expression, including programmed cell death-1 (PD-1),  
143 T-cell immunoglobulin and mucin domain-containing molecule-3 (TIM-3), cytotoxic T  
144 lymphocyte-associated antigen-4 (CTLA-4) and lymphocyte activation gene-3 (LAG-3), has been  
145 described as being a hallmark of dysfunctional T cells and tumor progression (14). Drugs  
146 targeting immune-checkpoints, in particular the PD-1/PD-L1 pathway, can unleash anti-tumor  
147 immunity and mediate durable cancer regression (15–17). Nevertheless, these new treatments are  
148 not efficient in all patients and identifying factors that predict clinical response to these therapies  
149 remains a challenge. In melanoma, an association between high PD-L1 expression and clinical  
150 response to pembrolizumab had been reported (18). However, patients with PD-L1-negative  
151 tumors may also achieve durable responses. In NSCLC, several efforts have also been made  
152 recently to more accurately identify patients that would respond to checkpoint therapy. The focus  
153 here has largely been on the identification of predictive markers for response to anti-PD-1, such  
154 as tumor mutational burden (TMB) (19), PD-L1 expression by tumor cells (20, 21), and gene  
155 signature reflecting adaptive immunity (22).

156 In this context, the present study investigated the potential impact of COPD on the  
157 immune microenvironment of NSCLC and, thus, on patients' outcome. Our work reveals that  
158 COPD severity is positively correlated with the level of CD8 TIL exhaustion. In agreement, we  
159 observed a complete loss of CD8 T cell-associated favorable clinical outcome in COPD<sup>+</sup> patients.  
160 Finally, data obtained on 39 advanced-stage NSCLC patients treated by an anti-PD-1 might  
161 suggest a higher sensitivity to this treatment in patients with COPD.

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163

164

165 **Methods**

166 **Patients**

167 A retrospective consecutive cohort of 435 NSCLC untreated patients seen between June  
168 2001 and December 2005 at the department of Thoracic Surgery of Hôtel-Dieu hospital (Paris,  
169 France) was used to study by immunohistochemistry the immune composition of the tumor  
170 microenvironment. A second cohort of fresh tumor samples, distant non-tumoral lung samples  
171 and peripheral blood were obtained from 50 patients with untreated NSCLC who underwent  
172 surgery between March 2014 and December 2015. These samples were used to perform flow  
173 cytometry experiments. A third cohort of 39 patients with advanced-stage NSCLC receiving an  
174 anti-PD-1 antibody (nivolumab) was used to assess the effectiveness of this treatment according  
175 to COPD status. Additional details are provided in the online supplements.

176

177 **COPD assessment**

178 The Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria were used to  
179 assess the presence of COPD and to evaluate the severity of airflow obstruction (23). Additional  
180 details are provided in the online supplements.

181

182 **Immunohistochemistry**

183 Serial section of paraffin-embedded NSCLC tumors were stained as previously described  
184 (24). Additional details are provided in the online supplements.

185

186 **Methods for cell Quantification**

187 Calopix software (Tribvn) was used to count CD66b<sup>+</sup> and CD68<sup>+</sup> cells in the whole tumor  
188 section; CD8 T cells were counted separately in the tumor nests and in tumor stroma. DC-Lamp<sup>+</sup>

189 cells were counted manually in the whole tumor section. The proportion of PD-L1<sup>+</sup> cells among  
190 tumor cells was determined manually by at least two independent observers (JB, HO or DD). The  
191 positivity threshold was fixed at  $\geq 1\%$ . Additional details are provided in the online supplements  
192 (Table E1).

193

#### 194 **Flow cytometry**

195 Multiple stainings on isolated mononuclear cells from Tumor, NT and Blood were  
196 performed using various antibodies (see Table E2 and Figure E1), as previously described (24)  
197 Additional details are provided in the online supplements.

198

#### 199 **Genomic DNA extraction and Illumina-based whole-exome sequencing.**

200 Genomic DNA from tumors was isolated from formalin-fixed paraffin-embedded blocks  
201 using Maxwell® 16 FFPE Tissue LEV DNA Purification Kit (Promega), according to the  
202 manufacturer's instructions. DNA whole exome sequence (WES) data were sequenced on the  
203 Novaseq 6000 platform (25). Additional details are provided in the online supplements.

204

#### 205 **Statistics**

206 Categorical data were compared using Chi-square tests or Fisher exact tests, as  
207 appropriate, while they were compared according to COPD stages using exact Cochran-Armitage  
208 trend test. For log-rank tests, the prognostic value of continuous variables was assessed by a  
209 quartile stratification. For Cox proportional-hazard models, immune cell densities were log-  
210 transformed. Multivariate analysis for OS was adjusted for age, gender, vascular emboli, smoking  
211 history and stratified on the tumor stage.

212 In flow cytometry experiments, according to data distribution, a parametric test (ANOVA,  
213 student's t test) or a non-parametric test (Kruskal-Wallis, Mann-Whitney), with appropriate post-  
214 hoc comparisons, was used to compare quantitative variables across the different groups.  
215 Correlations between quantitative parameters were assessed by using the Spearman test.  
216 Additional details are provided in the online supplements.

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235 **Results**

236 **COPD does not affect immune cell density in NSCLC tumor microenvironment.**

237 We first investigated the impact of COPD on the composition of the tumor immune  
238 microenvironment in a retrospective cohort of 435 NSCLC patients. Among them, 45% had  
239 COPD, and in the COPD<sup>+</sup> group, 29% had a COPD GOLD stage I (COPD<sup>+</sup> I), 60% a COPD  
240 GOLD stage II (COPD<sup>+</sup> II) and 11% a COPD GOLD stage III (COPD<sup>+</sup> III) (Table 1 and E3). The  
241 mean age, the percentages of male and of smokers were higher for COPD<sup>+</sup> than COPD<sup>-</sup> patients  
242 (Table 1). Coexisting COPD was associated with significant worse survival only for NSCLC  
243 stage I patients (Figure E2). Because of the small number of COPD<sup>+</sup> III patients, the COPD<sup>+</sup> II  
244 and COPD<sup>+</sup> III groups were merged for most of the subsequent analyses. COPD<sup>+</sup> and COPD<sup>-</sup>  
245 patients did not differ in density of neutrophils (CD66b<sup>+</sup> cells), macrophages (CD68<sup>+</sup> cells),  
246 mature DCs (DC-Lamp<sup>+</sup> cells) and CD8 T cells in tumor nests (CD8<sub>Tu</sub>) and in stroma (CD8<sub>s</sub>),  
247 regardless of GOLD stage (Figure E3).

248

249 **Absence of immune cell prognostic value in NSCLC patients with moderate to severe**  
250 **COPD**

251 To indirectly investigate whether the functionality of immune cells in the tumor  
252 microenvironment could be modified by coexisting COPD, we first determined their impact on  
253 patient survival according to COPD status and GOLD stage (Figure 1) (Table E4 and E5). In the  
254 whole retrospective cohort and in the COPD<sup>-</sup> group, univariate Cox-regression analysis showed  
255 that CD8<sub>Tu</sub>, CD8<sub>s</sub> and DC-Lamp<sup>+</sup> cell densities were all associated with favorable prognostic  
256 value, while neutrophil and macrophage density had no impact on patient survival (Figure 1A and  
257 B). Strikingly, CD8<sub>Tu</sub> cell density did not affect patient survival in COPD<sup>+</sup> patients (Figure 1C).  
258 Furthermore, CD8<sub>Tu</sub>, CD8<sub>s</sub> and DC-Lamp<sup>+</sup> cell densities were not significantly associated with  
259 improved survival in COPD<sup>+</sup> II-III patients (Figure 1D).

260 Then, patients were stratified by quartiles of CD8<sub>Tu</sub> cell density. In COPD<sup>-</sup> patients, CD8<sub>Tu</sub>  
261 cell density was associated with longer overall survival (OS) as soon as the 2<sup>nd</sup> quartile was  
262 reached (Figure 1E), whereas in COPD<sup>+</sup> and COPD<sup>+</sup> II-III patients the survival curves for all  
263 quartiles merged together (Figure 1F and G). DC-Lamp<sup>+</sup> cell and CD8<sub>S</sub> cell densities were not  
264 associated with significant prognostic value in COPD<sup>+</sup> II-III patients (Figure E4). Multivariate  
265 Cox-regression analysis adjusted for age, gender, vascular emboli, smoking history and stratified  
266 on tumor stages, highlighted the absence of CD8 T cell prognostic value for the stroma and tumor  
267 nests in COPD<sup>+</sup> patients (Figure 1H-J). Together, these data might suggest that the protective  
268 impact of a high adaptive immune cell infiltration in NSCLC is altered in COPD<sup>+</sup> patients and  
269 identify CD8<sub>Tu</sub> cells as the most affected population.

270

271

### 272 **TIL exhaustion, identified by PD-1/TIM-3 co-expression, is correlated with COPD severity**

273 Based on above results, we investigated whether effector functions of TILs were altered  
274 in COPD<sup>+</sup> patients using a prospective cohort of 50 NSCLC patients (Figure 2 and E5) (Table E6  
275 and E7). Regardless of COPD status, within the tumor tissue (Tumor), the proportion of CTLA-  
276 4<sup>+</sup>, LAG-3<sup>+</sup>, PD-1<sup>+</sup> and TIM-3<sup>+</sup> cells among CD4 (Figure E5A) and CD8 T cells (Figure 2A and  
277 B) was consistently higher than in the other anatomical sites (blood and non-tumor distal lung  
278 tissue (NT)). A marked increased of CD4<sup>+</sup> FoxP3<sup>+</sup> regulatory T cells (Treg) frequency among  
279 total CD4 T cells in Tumor was also observed (Figure E6A and B). Regarding cytokine secretion,  
280 the frequency of CD4 (Figure E5B) and CD8 T cells (Figure 2C and D) positive for Granzyme B,  
281 TNF- $\alpha$ , IFN- $\gamma$  and IL-17 was lower in Tumor than in NT. As shown by the correlation matrix  
282 exposed on Figure 2E, among CD8 TILs, PD-1 and TIM-3 expression was strongly positively  
283 correlated, as was the frequency of IFN- $\gamma$ <sup>+</sup> and TNF- $\alpha$ <sup>+</sup> cells. Remarkably, CD8 TILs co-

284 expressing PD-1 and TIM-3 were restricted to Tumor (Figure 2F), and for this cell subset only,  
285 there was a significant inverse correlation with both IFN- $\gamma$  and TNF- $\alpha$  secretion (Figure 2G).  
286 Overall, similar results were observed regarding CD4 TILs (Figure E5C and D). However, less  
287 CD4 TILs coexpressed PD-1/TIM-3 (Figure E5D), and the relationship between cytokine  
288 secretion and PD-1/TIM-3 coexpression was weaker (Figure E5C and E).

289         Based on above results, we investigated the link between COPD and TIL exhaustion. In  
290 COPD, airflow obstruction severity is inversely correlated with the Forced Expiratory Volume in  
291 1 second expressed as a percentage of normal predicted values (FEV1% predicted) (see Method  
292 section). Remarkably, FEV1% predicted was inversely correlated with the proportion of CD8  
293 TILs expressing PD-1 and co-expressing PD-1/TIM-3 in COPD<sup>+</sup> patients only (Figure 3A). In  
294 agreement, in COPD<sup>+</sup> patients, FEV1% predicted was positively correlated with the proportion of  
295 CD8 TILs secreting TNF- $\alpha$  and IFN- $\gamma$  (Figure 3A). Regarding CD4 TILs, only IFN- $\gamma$  was  
296 positively correlated with FEV1% predicted (Figure E7A). Interestingly, frequency of TIM-3<sup>+</sup>,  
297 PD-1<sup>+</sup> and TIM-3<sup>+</sup>/PD-1<sup>+</sup> cells among CD4 and CD8 TILs was higher in COPD<sup>+</sup> II-III patients  
298 than in COPD<sup>-</sup> patients (Figure 3B) (Figure E7B). The proportion of Treg among CD4 TILs was  
299 not different according to patients' COPD status (Figure E7C). Overall, these results demonstrate  
300 that COPD severity is strongly correlated with TIL exhaustion, and that this association is more  
301 pronounced for CD8 TILs.

302

### 303 **Strong correlation between CD8<sub>Tu</sub> cell density and CD8 TIL exhaustion: a phenomenon** 304 **exacerbated in COPD<sup>+</sup> patients**

305         An association between CD8 TIL exhaustion (PD-1<sup>+</sup> cell frequency) and the immune  
306 composition of the tumor microenvironment (density of CD8<sup>+</sup> T cells) was recently reported in  
307 colorectal cancer (26). We investigated this interrelation in our prospective cohort and then

308 studied the impact of COPD. First, CD8 TIL exhaustion (based on PD-1 and TIM-3 expression)  
309 and cytokine secretion were only linked to CD8<sub>Tu</sub> cell and CD8<sub>S</sub> cell densities (Figure E8A).  
310 Regarding CD4 TILs, none of the immune cell densities studied was associated with their  
311 exhaustion, and only their cytokine secretion was slightly inversely correlated with CD8<sub>S</sub> cell  
312 density (Figure E7D). Due to the strong association between CD8 TIL exhaustion and their  
313 density in the tumor nests, we then focused our analysis on CD8 TILs. Importantly, CD8<sub>Tu</sub> cell  
314 density and CD8 TIL exhaustion were more strongly associated in COPD<sup>+</sup> patients than in  
315 COPD<sup>-</sup> patients (Figure 3C) and (Figure E8B and C).

316 To confirm these results, median CD8<sub>Tu</sub> cell density was used to separate patients into two  
317 groups according to a Low (Figure 3D) or a High (Figure 3E) CD8<sub>Tu</sub> cell density. In the CD8<sub>Tu</sub><sup>Low</sup>  
318 group, the level of CD8 TIL exhaustion did not differ according to COPD status (Figure 3D). In  
319 the CD8<sub>Tu</sub><sup>High</sup> group, the frequencies of CD8 TILs expressing TIM-3 and co-expressing  
320 PD-1/TIM-3 were significantly higher in COPD<sup>+</sup> patients than in COPD<sup>-</sup> patients (Figure 3E).  
321 Overall, CD8 TIL exhaustion was restricted to highly CD8 T cell infiltrated tumors and this  
322 phenomenon was exacerbated in COPD<sup>+</sup> patients.

323

324

325 **PD-L1 expression by malignant cells is associated with shorter survival only in CD8<sub>Tu</sub><sup>High</sup>**  
326 **COPD<sup>+</sup> patients**

327 The strong impact of immunosuppression on tumor burden is based on TIL exhaustion,  
328 but also on concomitant mechanisms that malignant cells develop to avoid immune surveillance.  
329 The most-studied mechanism is probably PD-L1 expression by malignant cells (Figure E9A-D).  
330 No difference of PD-L1 expression by tumor cells was observed according to patients' COPD  
331 status and Gold stages (retrospective cohort) (Figure E9F). Consistent with previous studies, we



332 found that high CD8 T cell density is associated with high PD-L1 expression by tumor cells  
333 (Figure E9G) (27, 28). The frequency of tumor cells expressing PD-L1 was also higher, but to a  
334 lesser extent, in neutrophil<sup>High</sup>, macrophage<sup>High</sup> and DC-Lamp<sup>High</sup> groups. Additionally, among  
335 these highly infiltrated groups, PD-L1 expression was similar between COPD<sup>-</sup> and COPD<sup>+</sup>  
336 patients (Figure E9G).

337 We then investigated whether coexisting COPD modified the prognostic value of PD-L1  
338 expression by tumor cells. Whatever the group of patients considered, PD-L1 expression was not  
339 associated with significant prognostic value (Figure 4A and B). Since PD-L1 expression was  
340 strongly linked to CD8 T cell density (Figure E9G), we then deciphered the prognostic value of  
341 PD-L1 according to a High/Low CD8<sub>Tu</sub> cell density, and also to patient COPD status. For  
342 CD8<sub>Tu</sub><sup>Low</sup> groups, PD-L1 expression was not associated with significant prognostic value in  
343 COPD<sup>-</sup> or in COPD<sup>+</sup> patients (Figure 4C and D). Interestingly, for CD8<sub>Tu</sub><sup>High</sup> groups, PD-L1  
344 expression did not affect survival for COPD<sup>-</sup> patients (Figure 4E), but was associated with a  
345 reduced OS for COPD<sup>+</sup> patients (Figure 4F). Moreover, in COPD<sup>-</sup> patients, the prognostic value  
346 of CD8<sub>Tu</sub> and of CD8<sub>S</sub> cell density was similar whether tumor cells expressed PD-L1 or not  
347 (Figure 4G). Remarkably, for COPD<sup>+</sup> patients, CD8<sub>Tu</sub> and CD8<sub>S</sub> cell densities were associated  
348 with extended OS for those with PD-L1<sup>-</sup> tumors, while these prognostic values were not observed  
349 in the PD-L1<sup>+</sup> groups (Figure 4H). Finally, these results were confirmed in subgroups of patients  
350 defined by a cut-off of PD-L1<sup>+</sup> tumor cell frequency  $\geq 5\%$  (Figure E10A and B) and  $\geq 10\%$   
351 (Figure E10C and D).

352

353 **Anti-PD-1 antibody (nivolumab) efficacy in advanced-stage NSCLC patients according to**  
354 **coexisting COPD and to smoke exposure**

355 We investigated the impact of COPD on the effectiveness of an anti-PD-1 antibody from a  
356 cohort of 39 patients with advanced-stage NSCLC receiving nivolumab (Table E8). The  
357 percentage of smokers and the number of pack-years were higher for COPD<sup>+</sup> than COPD<sup>-</sup>  
358 patients, while no significant differences were observed between the two groups of patients  
359 regarding NSCLC subtypes, the duration of follow-up, the age and the gender (Table E8). At the  
360 completion of the study, a significantly longer progression-free survival (PFS) (Figure 5A and B)  
361 and a higher percentage of patients still alive (Table E8 and E9) (Figure 5C) were observed in the  
362 COPD<sup>+</sup> group. However, we did not observe any impact of COPD severity, assessed using the  
363 FEV1 % predicted, on nivolumab efficacy (Table E9).

364 In addition, it was previously shown that the efficacy of pembrolizumab, another anti-PD-  
365 1 antibody, was greater in patients with a smoking-associated mutational signature or with a  
366 higher nonsynonymous mutation burden in tumor (19). Consequently, we investigated whether  
367 the increased PFS seen with nivolumab in COPD<sup>+</sup> patients was linked to the higher smoke  
368 exposure observed in this group (Table E8). In the whole cohort, a smoke exposure >30 pack-  
369 years was associated with a better PFS and OS (Table E9), while in non-COPD patients, smoke  
370 exposure >30 pack-years was not associated significantly with a better PFS or OS (Table E9)  
371 (Figure 5D and F). Remarkably, in the COPD<sup>+</sup> group, a smoke exposure >30 pack-years was  
372 associated with a better PFS and also with a dramatic improvement of OS (Table E9) (Figure 5E  
373 and G). Regarding PFS, these results were confirmed when smoke exposure was assessed using  
374 number of pack-years as a continuous variable (Table E9).

375 A strong relationship between tobacco smoke exposure and the number of somatic  
376 mutations was previously reported in NSCLC (29). Consequently, we tried to investigate whether  
377 the number of nonsynonymous mutations per megabase (TMB) differ according to  
378 patients' COPD status, by performing whole exome sequencing (WES) experiments. Among the

379 22 patients for whom enough DNA was available (Figure 5C), we did not observe any difference  
380 regarding the TMB in COPD<sup>+</sup> patients compared to non-COPD patients (Figure E11A).  
381 Remarkably, the number of pack-years was significantly correlated with the TMB only in non-  
382 COPD patients (Figure E11C-E). Additionally, it has been shown that *TP53* and/or *KRAS*-  
383 mutated tumors, two mutations strongly associated with tobacco smoke exposure (30, 31), had a  
384 better response to PD-1 blockade (32). Among the 31 patients who had a molecular interrogation  
385 of their tumor before starting anti-PD-1 treatment (Figure 5C), using next-generation sequencing  
386 (NGS), we did not detect a differential distribution of *TP53* and/or *KRAS*-mutated tumors  
387 according to patients' COPD status (Figure E11B).

388 Our preliminary data might suggest a differential link between smoking history and  
389 response to nivolumab, but also between TMB and smoking history, in COPD<sup>+</sup> patients vs non-  
390 COPD patients. Consequently, we investigated whether COPD and tobacco had a synergistic  
391 impact on CD8 TIL exhaustion and on immune cell prognostic value. First, in the prospective  
392 cohort, the number of pack-years was positively correlated with the proportion of CD8 TILs co-  
393 expressing PD-1/TIM-3 in COPD<sup>+</sup> patients only (Figure E12A). Moreover, a higher CD8 TILs  
394 exhaustion was observed in COPD<sup>+</sup> group compared with COPD<sup>-</sup> group, in patients with a  
395 number of pack-years > 60 (Figure E12B). Secondly, in our retrospective cohort of 435 NSCLC  
396 patients, we investigated whether immune cell prognostic value (CD8<sub>Tu</sub>, CD8<sub>S</sub> and DC-Lamp<sup>+</sup>  
397 cells) was impacted by a strong smoke exposure (>30 pack-years). In heavy smokers, immune  
398 cell prognostic value was stronger in non-COPD patients (Figure E12C), than the one observed in  
399 Figure 1B. Conversely, in COPD<sup>+</sup> patients, the CD8<sub>S</sub> cell prognostic value was not significant in  
400 heavy smokers, and was completely absent for CD8<sub>Tu</sub> cells (HR:1.01,  $p=0.948$ ) (Figure E12D).  
401 Altogether, NSCLC patients with COPD, a group characterized by a complete loss of CD8 T cell-

402 associated favorable clinical outcome in heavy smokers probably due to their marked exhaustion,  
403 also showed a longer PFS after nivolumab treatment.

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405

406 **Discussion**

407 Our main objective was to evaluate the potential impact of COPD on the immune  
408 contexture of NSCLC. First, immune cell densities did not differ according to the COPD status of  
409 the patients. Immune cell recruitment into the malignant lesion is probably driven by tumor cells  
410 according to their immunogenicity linked to their mutational burden, thereby attenuating the  
411 impact of coexisting COPD. Co-occurring genetic alterations in *KRAS*-mutant lung  
412 adenocarcinoma, were associated with different tumor immune patterns, and could be a first  
413 argument supporting this hypothesis (33). However, our study showed higher TIL exhaustion in  
414 the COPD<sup>+</sup> II-III group, an impaired protective impact of immune cells in patients with COPD,  
415 and identified CD8 TILs as the most affected population.

416 The characteristics of the NSCLC immune contexture linked to CD8 TIL exhaustion are  
417 not completely defined, and the role of COPD in this phenomenon is not completely elucidated.  
418 We identified that CD8 TIL exhaustion was restricted to highly CD8 T cell infiltrated tumors,  
419 and these findings were exacerbated in COPD<sup>+</sup> patients. Interestingly, PD-1 expression, which is  
420 upregulated on T cells after TCR ligation (34), is also upregulated in activated T cells by IL-6  
421 through STAT3-dependent mechanisms (35). Accordingly, high frequency of exhausted TILs  
422 observed in COPD<sup>+</sup> II-III patients could be driven in part by the increased amounts of IL-6  
423 previously reported in the sputum of COPD<sup>+</sup> patients (36). It is also conceivable that when a  
424 tumor forms near an emphysematous/inflammatory lesion, the surrounding inflammation,  
425 including numerous cytokines (IL-6, TNF- $\alpha$ , IL-1 $\beta$ ) and chemokines (CXCL8, CXCL1) (37)  
426 modify autocrine and paracrine interactions between malignant cells and infiltrating leucocytes.  
427 Interestingly, *ex vivo* infections with influenza virus of lung resections showed an impaired  
428 antiviral function of CD8<sup>+</sup> T cells in COPD<sup>+</sup> patients compared to non-COPD patients, through an  
429 up-regulation of PD-1 expression. Moreover, if these PD-1 expressing CD8 T cells, coming from

430 the non-tumor distal lung tissue, are overrepresented in the tumor immune microenvironment of  
431 COPD<sup>+</sup> patients, they might participate in the deviation of the anti-tumor immune response  
432 observed in patients with COPD (38). Nevertheless, orthogonal approaches, including gene  
433 expression analysis related to the immune response in cancer, are required to precisely identify  
434 the characteristics of the inflammation disrupting the tumor immune contexture of patients with  
435 NSCLC and COPD.

436 In agreement with other studies (17, 27, 39), we observed that PD-L1 expression by  
437 NSCLC tumor cells highlights the presence of an active tumor immune microenvironment in lung  
438 cancer, independently of COPD. This is probably due to the fact that cancer cells may upregulate  
439 PD-L1 expression in response to IFN- $\gamma$  secretion by TILs (39). Studies of the prognostic value of  
440 PD-L1 expression in patients with NSCLC has yielded inconsistent data (40–44). These  
441 conflicting results could be due to the fact that the amount of CD8 TILs and patients' COPD  
442 status had not been taken into account. In our study, the prognostic value of PD-L1 expression  
443 was restricted to COPD<sup>+</sup> patients belonging to the CD8<sub>Tu</sub><sup>High</sup> group. It probably reflects the  
444 effectiveness of mechanisms that cancer cells develop to avoid immune surveillance in a  
445 subpopulation of patients characterized by a strong CD8 TIL exhaustion. In CD8<sub>Tu</sub><sup>Low</sup>, the lack of  
446 PD-L1 prognostic value was probably linked to the lowest impact of the PD-1/PD-L1 pathway on  
447 a weakly active anti-tumor immune response. In this situation, PD-L1 expression is probably  
448 driven more through oncogenic pathways, including inactivation of STK11/LKB1 (45) or loss of  
449 function of the tumor suppressor PTEN (46), and not associated to a strong PD-1 expression by  
450 CD8 TILs. Moreover, immune cell prognostic value was completely absent for tumors from  
451 COPD<sup>+</sup> patients expressing PD-L1, probably because the level of TIL exhaustion was higher in  
452 this group. Interestingly, in melanoma, preexisting CD8 TILs in tumor microenvironment were  
453 required for tumor regression after treatment with pembrolizumab (27), and CD8 TILs

454 coexpressing PD-1/CTLA-4 had been proposed as a biomarker to predict response to anti-PD-1  
455 (47). Altogether, for NSCLC patients with moderate to severe COPD, our results support an  
456 increased predictive potential of PD-L1 expression by tumor cells for the response to checkpoint  
457 inhibitors targeting the PD-1/PD-L1 pathway (48).

458         In accordance with this assumption, our preliminary data obtained in a cohort of 39  
459 nivolumab treated NSCLC patients showed a longer PFS in patients with coexisting COPD (19).  
460 Nevertheless, we did not identify any impact of COPD severity on the response to anti-PD-1. It is  
461 conceivable that we had not enough patients to observe this kind of effect. Moreover, other  
462 confounding factors not explored in our work could explain the lack of association between  
463 COPD severity and the response to nivolumab, including among others, CD8 TIL density and  
464 PD-L1 expression by tumor cells. Remarkably, at the time of publication, the study from *Mark et*  
465 *al*, also observed that the presence of COPD was associated with longer progression free survival  
466 interval in patients treated with anti-PD-1 or anti-PD-L1 (49). However, a smoking-associated  
467 mutational signature had previously been suggested to signal a better response to immunotherapy.  
468 Since, tobacco and COPD may be confounding factors in the response to nivolumab, we tried to  
469 investigate the interplay between these two factors, and if possible their respective impact. Our  
470 study suggests that the impact of tobacco on the response to nivolumab would be mainly  
471 observed in COPD<sup>+</sup> patients. In agreement with this assumption, when anti-PD1/PD-L1 impact  
472 was evaluated only in former smokers, *Mark et al* still observed a longer PFS and OS in COPD<sup>+</sup>  
473 patients (49). We also showed a stronger impact on patients' survival of the immune  
474 microenvironment in heavy smokers without COPD. In this situation, tumors are probably more  
475 immunogenic and the presence of a strong specific adaptive anti-tumor immune response even  
476 more important for patients' survival. Interestingly, in COPD<sup>+</sup> patients, the number of pack-years  
477 was positively correlated with the level of CD8 TIL exhaustion, and the impact of CD8<sub>Tu</sub> cell

478 density on patients' survival was completely absent in heavy smokers. Altogether, the higher  
479 nivolumab efficacy observed in COPD<sup>+</sup> patients, probably reflects the effectiveness of PD-1  
480 blockers to unleash anti-tumor CD8 T cell response in a subpopulation of patients characterized  
481 by strong CD8 TIL exhaustion.

482 Finally, we tried to investigate whether the longer PFS observed in nivolumab COPD<sup>+</sup>  
483 treated patients was linked to a higher TMB induced by a stronger tobacco smoke exposure.  
484 Unfortunately, we were able to perform such work only on 22 anti-PD-1 treated patients.  
485 However, our preliminary data are not in favor of a higher TMB in COPD<sup>+</sup> patients. Moreover,  
486 the frequency of *KRAS* and *TP53* mutations, two mutations strongly linked to tobacco smoke  
487 exposure (30, 31) that have been suggested as being associated with longer PFS in anti-PD-1  
488 treated patients (32) were not enriched in COPD<sup>+</sup> patients. In agreement with these results, our  
489 previous work did not detect a higher frequency of *TP53* or *KRAS* mutations in COPD<sup>+</sup> patients,  
490 again characterized by a stronger smoke exposure, in a cohort of 282 lung adenocarcinomas (31).  
491 Beyond the scope of the present work, studies using larger cohorts of patients are mandatory to  
492 precisely determine whether the longer PFS after PD-1 blockade observed in COPD<sup>+</sup> patients is  
493 mostly linked or not to their higher tobacco smoke exposure. Such studies will also allow to  
494 determine whether tobacco smoke exposure differentially impacts CD8 TIL exhaustion, TMB  
495 and response to PD-1 blockers, in COPD<sup>+</sup> patients vs non-COPD patients.

496 However, there were some limitations to our study. First, our cohort of anti-PD-1 treated  
497 patients is restricted to 39 patients, mainly because we needed a follow up of at least one year,  
498 combined to fully characterized respiratory functions. Secondly, the use of three cohorts  
499 inevitably increased the number of comparisons and the number of false discovery rate, but we  
500 tried to reduce this risk using the most appropriate statistical methodology and by applying  
501 appropriate adjustments for multiple comparisons. The impact of histological subtypes on our



502 findings was not fully analyzed, even it does not seem to impact our main results (Data not  
503 shown). In fact, we did not address this point to avoid an increase of multiple comparisons.  
504 Another limitation, inherent to this kind of work, is related to tissue heterogeneity, slide thickness  
505 and surface area covered. However, one strength of our study is that in immunochemistry  
506 experiments, we worked on whole tumor sections and not on tumor microarrays (TMA), with  
507 senior pathologists, using automates for staining, scanning and counting. All these precautions  
508 were intended to increase the accuracy and reproducibility of our data.

509         By deciphering the immune network of NSCLC, we pinpointed that the use of  
510 immunological biomarkers to evaluate patient prognosis (50) and to predict the response to  
511 therapy should definitively take into account the coexistence of COPD. This chronic  
512 inflammatory disease of the lung is not fully understood and its impact on tumor immune  
513 microenvironment functions and on response to immune checkpoint inhibitors will deserve  
514 additional experiments. We recommend that clinical trials should investigate whether CD8 TIL  
515 density and smoke exposure, together with coexisting COPD may identified the best responders  
516 to therapies targeting immune-checkpoints. Overall, our study emphasizes the need to consider  
517 the impact of coexisting chronic inflammation on the tumor immune microenvironment in other  
518 cancer types. In the era of precision medicine, such studies should extend the clinical success of  
519 immunotherapies in cancer.

520

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758 **Table 1**

Characteristics	<u>COPD<sup>-</sup></u>		<u>COPD<sup>+</sup></u>		P-value
	Number	(%)	Number	(%)	
<b>Subjects</b>	238	(55)	197	(45)	
<b>Gender</b>					
<b>Male/Female</b>	165/73	(69/31)	174/23	(88/12)	<b>&lt;0.0001*</b>
<b>Age (years)</b>					
<b>Mean ± SD</b>	61 ± 12		64 ± 9		<b>0.01</b>
<b>Smokers</b>	182	(78)	173	(91)	<b>0.0003*</b>
<b>Smoking history</b>					
<b>Pack-years ± SD</b>	36 ± 24		49 ± 24		<b>&lt;0.0001</b>
<b>Histological Subtypes</b>					
<b>ADC</b>	173	(73)	136	(69)	0.70*
<b>SSC</b>	53	(22)	50	(25)	
<b>Others</b>	12	(5)	11	(6)	
<b>pTNM stages</b>					
<b>I</b>	102	(43)	94	(48)	0.05*
<b>II</b>	58	(24)	59	(30)	
<b>III-IV</b>	78	(33)	44	(22)	
<b>Vascular Emboli</b>					
<b>Yes</b>	125	(53)	118	(60)	0.21*
<b>No</b>	98	(41)	65	(33)	
<b>ND</b>	15	(6)	14	(7)	
<b>Pleural Invasion</b>					
<b>Yes</b>	124	(52)	110	(56)	0.70 <sup>#</sup>
<b>No</b>	110	(46)	83	(42)	
<b>ND</b>	4	(2)	4	(2)	
<b>Lobectomy</b>	210	(88)	171	(87)	0.65*
<b>Pneumonectomy</b>	28	(12)	26	(13)	
<b>% FEV1/FVC</b>					
<b>Mean ± SD</b>	79 ± 5		60 ± 8		<b>&lt;0.0001</b>
<b>FEV1 (% of a predicted value)</b>					
<b>Mean ± SD</b>	90 ± 17		71 ± 17		<b>&lt;0.0001</b>

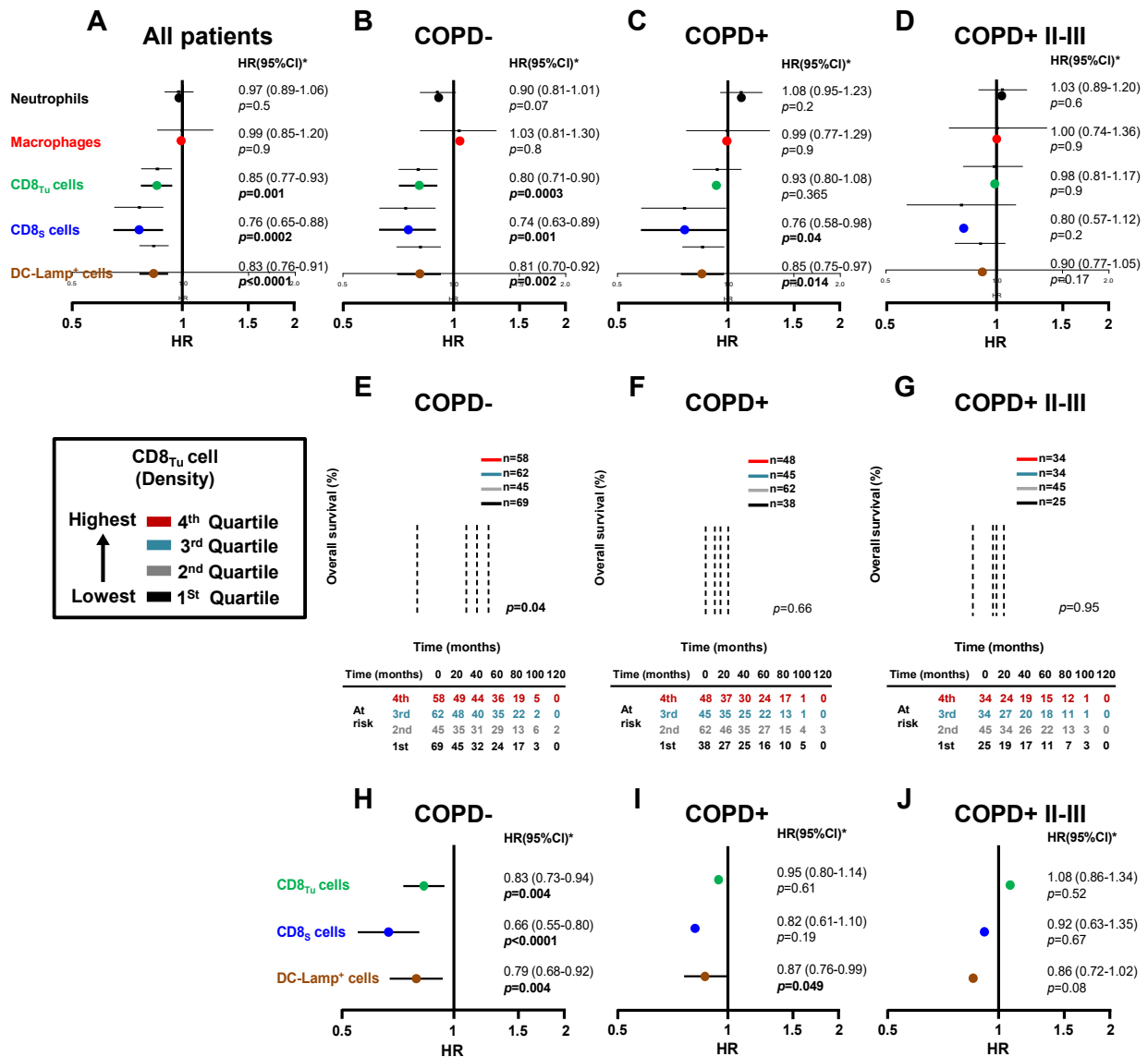
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760 **Table 1. Baseline characteristics of 435 patients with NSCLC (retrospective cohort).**

761 All variables were evaluated among the 435 patients with NSCLC. Quantitative data were  
762 compared between COPD<sup>-</sup> and COPD<sup>+</sup> patients by Student's t test. Categorical data were  
763 compared by chi-square test (\*) or Fisher exact test (#), as appropriate. P values <0.05 were  
764 considered statistically significant and appear in bold. Abbreviations: ADC, adenocarcinoma;  
765 SSC, squamous cell carcinoma; ND, Not determined.

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770 **Figure 1. Prognostic value of immune cell densities in NSCLC according to COPD**

771 **status (retrospective cohort). (A-D) Forest plots of univariate Cox-regression analysis**

772 **showing the impact of neutrophil (n=435), macrophage (n=435), CD8<sub>Tu</sub> (n=427), CD8s**

773 **(n=435) and DC-Lamp<sup>+</sup> cell (n=435) density on overall survival (OS) in the whole cohort (all**

774 **patients) (A), COPD<sup>-</sup> (B), COPD<sup>+</sup> (C) and COPD<sup>+</sup> II-III patients (D). (E-G) The quartile**

775 **method was used to stratify patients into four groups by density of CD8<sub>Tu</sub> cells, from the**

776 **lowest (1<sup>st</sup> quartile, black curves) to the highest density of CD8<sub>Tu</sub> cells (4<sup>th</sup> quartile, red**

777 **curves). Kaplan-Meier curves of OS according to CD8<sub>Tu</sub> cell density in COPD<sup>-</sup> (E), COPD<sup>+</sup>**

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778 (F) and COPD<sup>+</sup> II-III patients (G). The horizontal dashed lines represent the median  
779 survivals. (H-J) Forest plots of multivariate Cox-regression analysis showing the impact of  
780 CD8<sub>Tu</sub>, CD8s and DC-Lamp<sup>+</sup> cell density on OS adjusted for age, gender, vascular emboli,  
781 smoking history and stratified on the stage of the tumor, in COPD<sup>-</sup> (H), COPD<sup>+</sup> (I) and  
782 COPD<sup>+</sup> II-III patients (J). *P* values <0.05 were considered statistically significant and appear  
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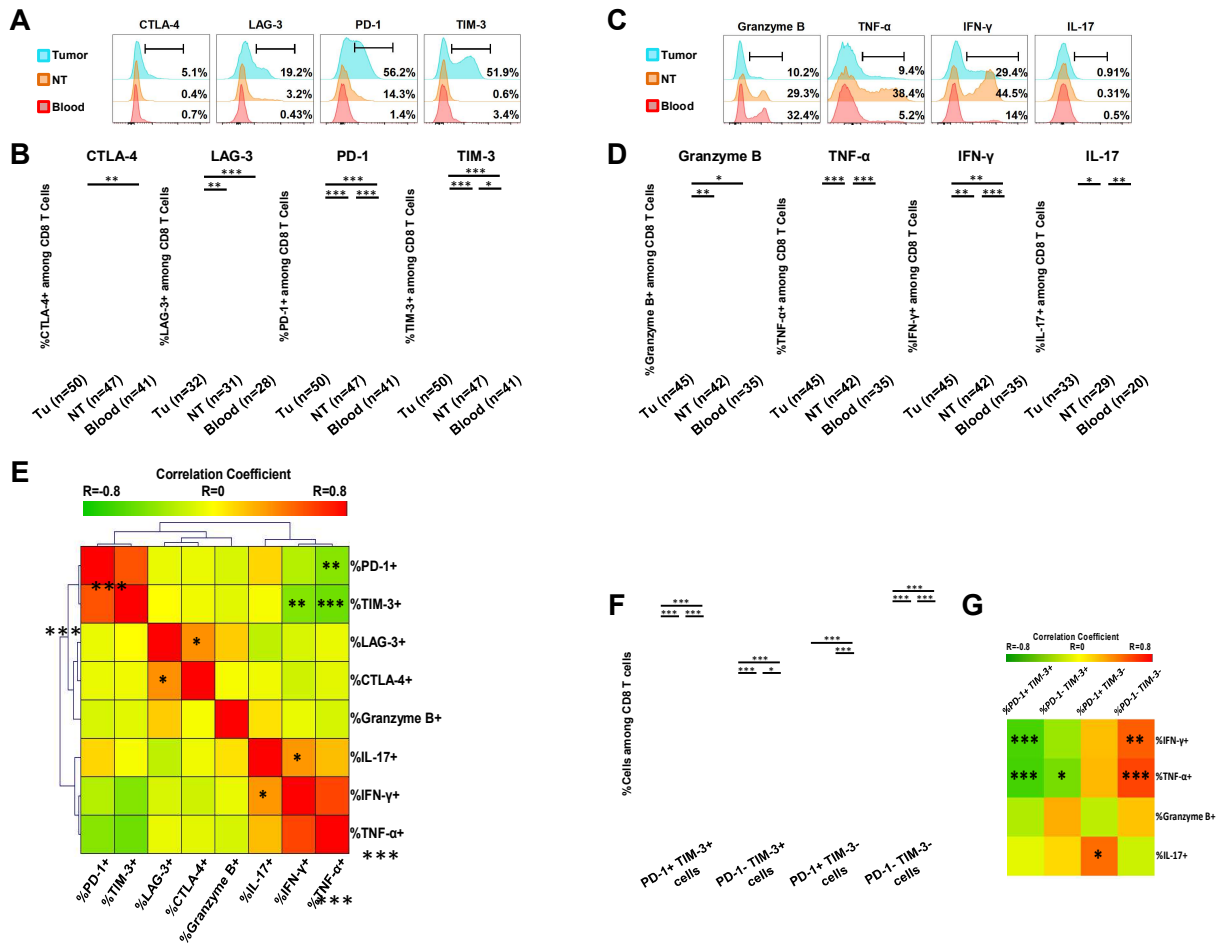
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805 **Figure 2. Characterization of CD8 tumor-infiltrating T lymphocytes (CD8 TILs) in**  
 806 **NSCLC (prospective cohort).**

807 (A) Histograms showing, for one representative patient, the frequency of CTLA-4<sup>+</sup>, LAG-3<sup>+</sup>,  
 808 PD-1<sup>+</sup> and TIM-3<sup>+</sup> cells among CD8 T cells in Tumor, NT and blood. (B) Frequency of  
 809 CTLA-4<sup>+</sup>, LAG-3<sup>+</sup>, PD-1<sup>+</sup> and TIM-3<sup>+</sup> cells among CD8 T cells in Tumor, NT and blood. (C)  
 810 Histograms showing, for one representative patient, the frequency of Granzyme B<sup>+</sup>, TNF-α<sup>+</sup>,  
 811 IFN-γ<sup>+</sup> and IL-17<sup>+</sup> cells among CD8 T cells in Tumor, NT and blood. (D) Frequency of  
 812 Granzyme B<sup>+</sup>, TNF-α<sup>+</sup>, IFN-γ<sup>+</sup> and IL-17<sup>+</sup> cells among CD8 T cells in Tumor, NT and blood.  
 813 (E) Spearman-correlation matrix of parameters studied by flow cytometry. Each colored

814 square illustrates the correlation between two parameters. Red color illustrates a strong  
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815 positive correlation, and green color illustrates a strong negative correlation. (F) Frequencies  
816 of PD-1<sup>+</sup> TIM-3<sup>+</sup>, PD-1<sup>+</sup> TIM-3<sup>-</sup>, PD-1<sup>-</sup> TIM-3<sup>+</sup>, PD-1<sup>-</sup> TIM-3<sup>-</sup> cells among CD8 T cells in  
817 Tumor (black circle, n=50), NT (grey circle, n=47) and blood (white circle, n=41). (G)  
818 Correlation between the frequencies of PD-1<sup>+</sup> TIM-3<sup>+</sup>, PD-1<sup>-</sup> TIM-3<sup>+</sup>, PD-1<sup>+</sup> TIM-3<sup>-</sup> and PD-  
819 1<sup>-</sup> TIM-3<sup>-</sup> cells among CD8 TILs and frequencies of Granzyme B<sup>+</sup>, IL-17<sup>+</sup>, TNF- $\alpha$ <sup>+</sup> and IFN-  
820  $\gamma$ <sup>+</sup> cells among CD8 TILs. In (B), (D) and (F) data are expressed as mean and an ANOVA or  
821 a Kruskal–Wallis test followed by an appropriate correction was applied based on Shapiro  
822 normality test. In (E) and (G) a spearman test was applied. \* $p$ <0.05, \*\* $p$ < 0.01 and  
823 \*\*\* $p$ <0.001.

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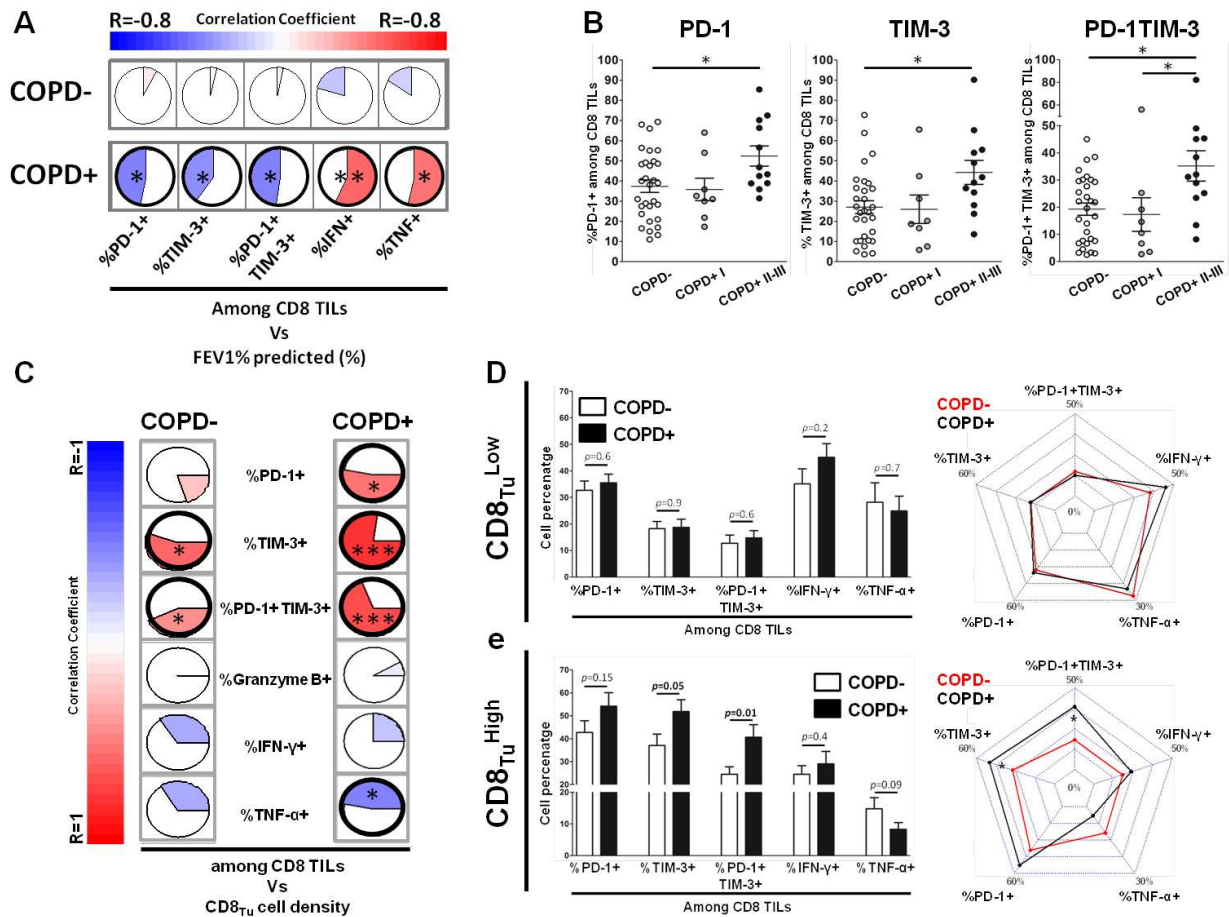
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840 **Figure 3**



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842 **Figure 3. CD8 TIL exhaustion according to the COPD status of the patients**  
 843 **(prospective cohort).**

844 (A-E) CD8 TIL characterization in COPD<sup>-</sup> (n=30), COPD<sup>+ I</sup> (n=8) and COPD<sup>+ II-III</sup> (n=12)  
 845 patients. (A) Graphical representation of Spearman correlations between the FEV1%  
 846 predicted and the frequencies of PD-1<sup>+</sup>, TIM-3<sup>+</sup>, PD-1<sup>+</sup> TIM-3<sup>+</sup>, Granzyme B<sup>+</sup>, IFN- $\gamma$ <sup>+</sup> and  
 847 TNF- $\alpha$ <sup>+</sup> cells among CD8 TILs, in COPD<sup>-</sup> patients and COPD<sup>+</sup> patients. Red and blue colors  
 848 indicate positive and negative correlations, respectively; the lighter is the color, the less  
 849 significant is the corresponding correlation. The filled fraction of the circle in each of the pie  
 850 charts corresponds to the absolute value of the associated Spearman correlation coefficient.  
 851 (B) Frequencies of PD-1<sup>+</sup>, TIM-3<sup>+</sup> and PD-1<sup>+</sup> TIM-3<sup>+</sup> cells among CD8 TILs. (C) Graphical  
 852 representation of Spearman correlations in COPD<sup>-</sup> and COPD<sup>+</sup> patients, between CD8<sub>Tu</sub> cell

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853 densities and flow cytometry data, including the frequencies of PD-1<sup>+</sup>, of TIM-3<sup>+</sup>, of PD-1<sup>+</sup>  
854 TIM-3<sup>+</sup>, Granzyme B<sup>+</sup>, IFN- $\gamma$ <sup>+</sup> and TNF- $\alpha$ <sup>+</sup> cells among CD8 TILs. Graphical representation  
855 is the same than in Figure 3A. **(D-E)** The median CD8<sub>Tu</sub> cell density was used to stratify  
856 patients into CD8<sub>Tu</sub><sup>Low</sup> **(D)** or CD8<sub>Tu</sub><sup>High</sup> **(E)** groups. **(D-E)** Histograms and radar plots showing  
857 the frequencies of PD-1<sup>+</sup>, TIM-3<sup>+</sup>, PD-1<sup>+</sup> TIM-3<sup>+</sup>, IFN-  $\gamma$ <sup>+</sup> and TNF- $\alpha$ <sup>+</sup> cells among CD8 TILs  
858 according to COPD status in CD8<sub>Tu</sub><sup>Low</sup> group and CD8<sub>Tu</sub><sup>High</sup> group. In **(A)** and **(C)** a Spearman  
859 Test was performed. In **(B)** data are expressed as mean  $\pm$  SEM and a parametric test  
860 (ANOVA with post-hoc Bonferroni correction) or a nonparametric test (Kruskal–Wallis test  
861 followed by a post-hoc Dunn's test) was applied based on Shapiro normality test. In **(D-E)**  
862 data are expressed as mean, and a parametric test (Student't test) or a nonparametric test  
863 (Mann Whitney test) was applied based on Shapiro normality test. \* $p$ <0.05, \*\* $p$ < 0.01 and  
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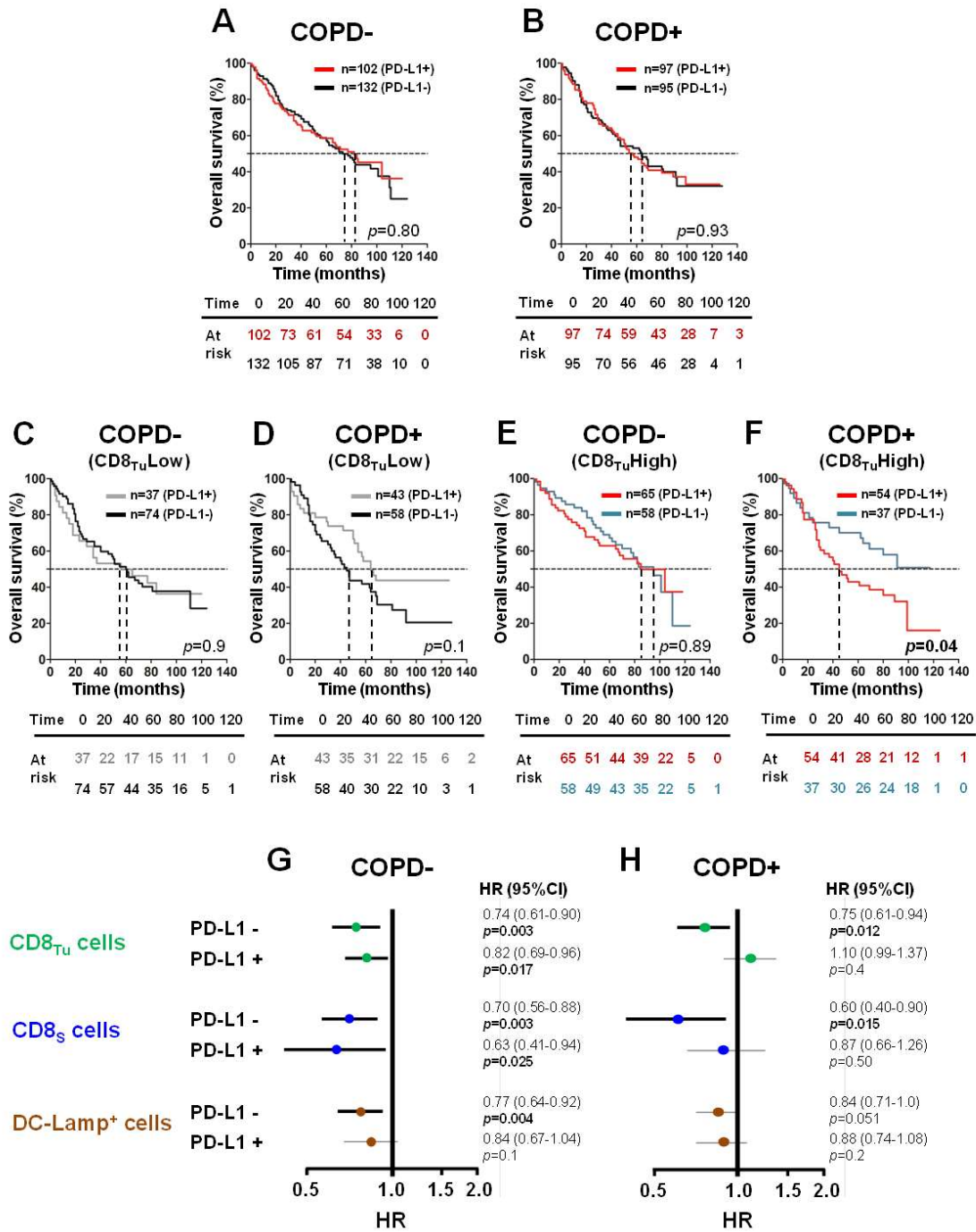
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881 **Figure 4. Immune cell prognostic value according to COPD status and PD-L1**  
 882 **expression by tumor cells (retrospective cohort).**

883 Kaplan-Meier curves of OS in NSCLC patients according to PD-L1 expression by tumor  
 884 cells, in COPD<sup>-</sup> patients (n=234) (A) and in COPD<sup>+</sup> patients (n=192) (B). (C) and (D)

885 Kaplan-Meier curves of OS in the CD8<sub>Tu</sub><sup>Low</sup> group according to PD-L1 expression by tumor

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886 cells in COPD<sup>-</sup> (n=111) (C) and COPD<sup>+</sup> patients (n=101) (D). (E) and (F) Kaplan-Meier  
887 curves of OS in the CD8<sub>Tu</sub><sup>High</sup> group according to PD-L1 expression by tumor cells in COPD<sup>-</sup>  
888 (n=123) (E) and COPD<sup>+</sup> patients (n=91) (F). (G) and (H) Forest plots of univariate Cox-  
889 regression analyses showing the impact of CD8<sub>Tu</sub> cell, CD8s cell and DC-Lamp<sup>+</sup> cell density  
890 on the OS according to PD-L1 expression by tumor cells, in COPD<sup>-</sup> patients (G) and in  
891 COPD<sup>+</sup> patients (H). *P* values <0.05 were considered statistically significant and appear in  
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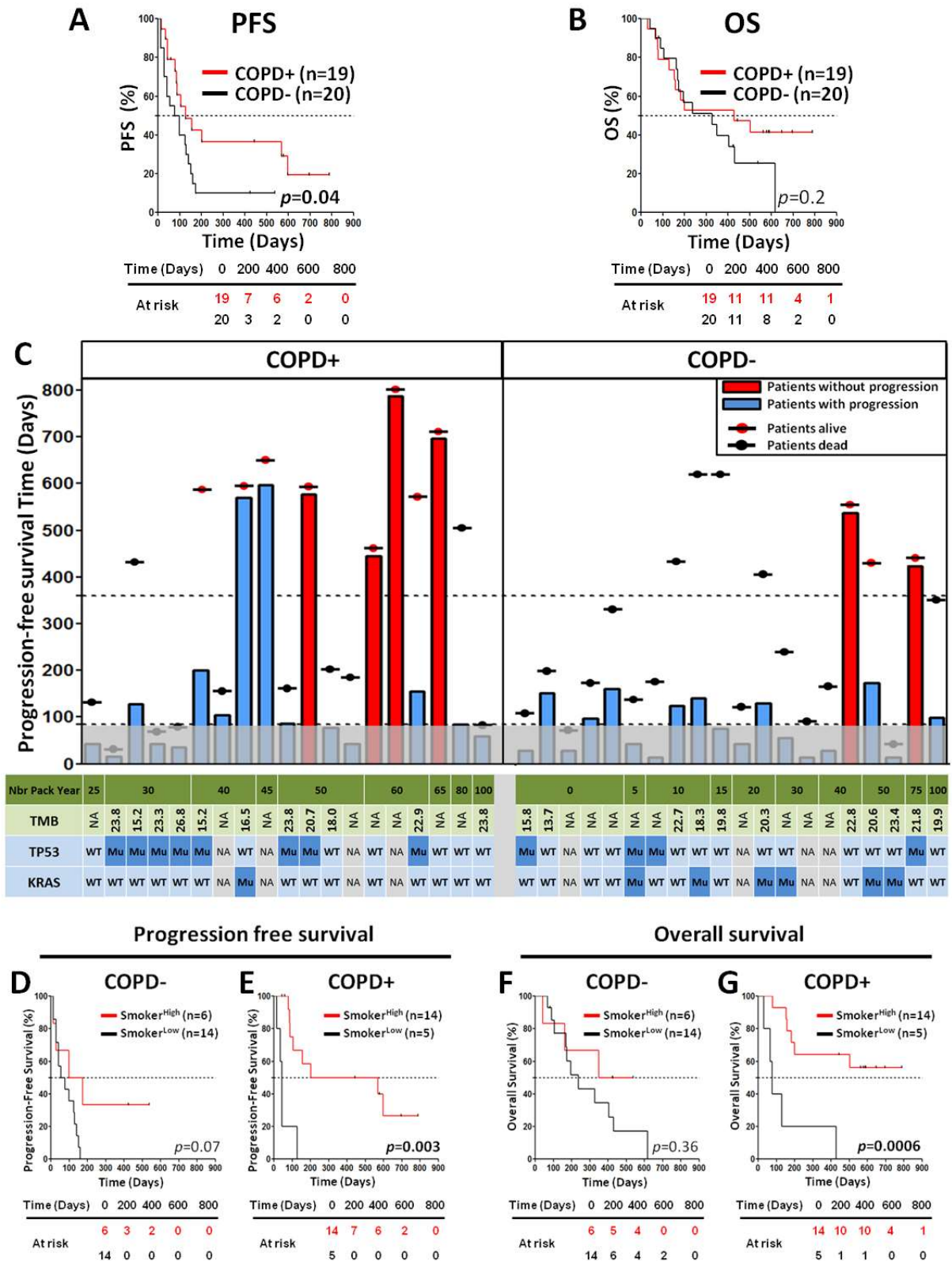
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913 Figure 5. Nivolumab efficacy in advanced-stage NSCLC patients according to a

914 coexisting COPD and to smoke exposure. (A-B) Kaplan-Meier curves of progression-free

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915 survival (PFS) and overall survival (OS) in COPD<sup>-</sup> (n=20) and COPD<sup>+</sup> patients (n=19).  
916 Progression-free survival was defined as the time from the start date of nivolumab treatment  
917 to the date of the first documented event of tumor progression. **(C)** Characteristics of the  
918 response to nivolumab treatment according to patients' COPD status and to smoking  
919 exposure. TMB, Tumor mutation burden expressed as number of nonsynonymous mutations  
920 per megabase and determined using whole exome sequencing experiments; Mu, Mutated-  
921 tumor; WT, Wild type-Tumor, NA; Not available. **(D-G)** Kaplan-Meier curves of  
922 progression-free survival **(D-E)** and of overall survival **(F-G)** in COPD<sup>-</sup> (n=20) **(D,F)** and  
923 COPD<sup>+</sup> (n=19) **(E,G)** patients according to smoking exposure. Patients were stratified into  
924 two groups, Smoker<sup>High</sup> (>30 pack-years) and Smoker<sup>Low</sup> (≤30 pack-years). Tick marks  
925 indicate censoring events. *P* values <0.05 were considered statistically significant and appear  
926 in bold.  
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