

ORIGINAL RESEARCH ARTICLE

**Title**

Tumor infiltrating lymphocyte density is associated with favorable outcome in advanced non-small cell lung cancer patients treated with immunotherapy

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## **ABSTRACT**

### **BACKGROUND**

The established role of morphological evaluation of tumor infiltrating lymphocytes (TIL) with immune checkpoint inhibitors (ICI) in non-small cell lung cancer (NSCLC) is unknown. We aimed to determine TIL association with outcome for ICI and for chemotherapy in advanced NSCLC.

### **METHODS**

Multicenter retrospective study of a nivolumab-cohort of 221 patients treated between Nov-2012 and Feb-2017, and a chemotherapy-cohort of 189 patients treated between Jun-2009 and Oct-2016. Patients with available tissue for stromal TIL evaluation were analyzed. High-TIL was defined as  $\geq 10\%$  density. The primary endpoint was overall survival (OS).

### **RESULTS**

Among the nivolumab-cohort, 64% were male, with median age of 63 years, 82.3% were smokers, 77% had performance status  $\leq 1$  and 63% had adenocarcinoma histology. High-TIL was observed in 22% patients, and associated with OS [HR 0.48; 95%CI 0.28-0.81] and PFS [HR 0.40; 95%CI, 0.25-0.64]. Median PFS was 13.0 months [95%CI, 5.0-not reached] with high-TIL vs. 2.2 months [95%CI, 1.7-3.0] with low-TIL. Median OS for high-TIL was not reached [95%CI, 12.2-not reached] vs. 8.4 months [95%CI, 5.0-11.6] in the low-TIL group. High-TIL was associated with ORR and DCR (both,  $P < .0001$ ). Among the chemotherapy-cohort, 69% were male, 89% were smokers, 86% had performance status  $\leq 1$ ; 90% had adenocarcinoma histology. High-TIL was seen in 37%. Median PFS and OS were 5.7 months [95%CI, 4.9-6.7] and 11.7 months [95%CI, 9.3-13.0] respectively, with no association with TIL.

### **CONCLUSIONS**

High-TIL was associated with favorable outcomes in a real-world immunotherapy cohort of NSCLC patients, but not with chemotherapy, suggesting that TIL may be useful selecting patients for immunotherapy.

**Keywords:**

Immunotherapy; immune checkpoint inhibitor; nivolumab; tumor infiltrating lymphocytes; TIL; lung cancer; non-small cell lung cancer; NSCLC; prognostic; biomarkers.

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**List of where and when the study has been presented in part elsewhere:**

- Gataa *et al*, ESMO Congress 2017; Poster (112P) Pathological evaluation of tumor infiltrating lymphocytes and the benefit of nivolumab in advanced non-small cell lung cancer (NSCLC), September 2017, *Annals of Oncology* 28(suppl\_5)
- Mezquita L *et al*, IASLC WCLC Conference 2019; Oral communication MA25.03 Tumor-Infiltrating Lymphocytes (TIL) and Outcomes with Immunotherapy (ICI) or Chemotherapy in Advanced NSCLC (aNSCLC) Patients; October 2019; *J Thorac Oncol*, Volume 14, Issue 10, Supplement, Pages S351–S352

## **HIGHLIGHT**

- High-TIL density ( $\geq 10\%$ ) by morphology, was associated with favorable outcomes in an immunotherapy cohort of NSCLC patients
- High-TIL density was not associated with outcomes in a chemotherapy cohort of 189 advanced NSLCC patients
- TIL may be useful selecting patients for immunotherapy.

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**Figures:**3

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## 1. INTRODUCTION

Immune checkpoint inhibitors (ICI) represent one of the most important milestones in the therapeutic strategy for cancer. In advanced non-small cell lung cancer (NSCLC), ICIs as single agent or in combination with chemotherapy or other ICIs have improved survival compared with chemotherapy alone, and are currently standard of care <sup>1</sup>.

However, the benefit derived from ICIs is restricted to a subset of patients. The development of biomarkers to identify candidates who may benefit from ICIs is currently a major challenge in this field. In NSCLC, PD-L1 expression is the only approved predictive factor identified to date <sup>1</sup>. This has some limitations, with objective response to ICIs described in the absence of expression. Other tumor-related biomarkers include tumor mutation burden and gene expression profiling, are still under investigation <sup>2,3</sup>.

Data about host-related biomarkers in the tumor microenvironment are rare. In NSCLC, the impact of specific immune cell subpopulations in the tumor microenvironment, such as effector T cells, has been correlated with ICI outcomes. The morphological assessment of tumor infiltrating lymphocytes (TIL), describing type, density, and location within tumor tissue, using hematoxylin & eosin (H&E) stained sections, has been described as a prognostic factor in several tumor types including NSCLC <sup>4,5,6</sup>.

The aim of this study was to determine the association between TIL and ICI outcomes in advanced NSCLC patients treated with monotherapy nivolumab, and to compare this with the association in chemotherapy cohort.

## 2. MATERIAL AND METHODS

In this retrospective study, two cohorts with advanced NSCLC, were studied: immunotherapy cohort (IC) and chemotherapy cohort (CC) (**Figure 1**). In the IC cohort, patients treated with nivolumab between November 2012 and February 2017 in three French centers. The CC, was retrospectively extracted from ICI-naïve advanced NSCLC cancer patients treated between June 2009 and October



2016 in the prospective MSN study (*NCT02105168*). Availability of archival tumor tissue was required. Clinical, pathological and molecular characteristics were retrospectively collected.

### **2.1. TIL density assessment**

TIL density was assessed semi-quantitatively by two pathologists (J.A and M.K.) using H&E stained sections from archival pretreatment tumor tissue samples, based on breast cancer TIL evaluation<sup>7</sup>. The percentage of tumor area containing stromal TIL was reported and high-TIL was defined as >10% based on the mean of the immunotherapy-cohort in preliminary results. This cut point was confirmed by the Log Rank maximization method.

### **2.2. PD-L1 immunohistochemistry**

PD-L1 expression was determined in the most recent cases, using a laboratory-developed test with clone 28-8 (Abcam) or E1L3N (Cell Signaling Technology), calibrated in comparison to the PD-L1 22C3 PharmDx assay. Tumor cell staining was scored by a lung pathologist and considered as positive if  $\geq 1\%$  of tumor cells exhibited membranous staining at any intensity.

### **2.3. Response assessment**

The primary endpoint was overall survival (OS). Secondary endpoints were progression-free survival (PFS), disease control rate (DCR) and overall response rate (ORR). Radiological assessments were performed per RECIST v1.1 or per the investigator's discretion, according to the routine of each center.

Progression-free survival (PFS) was defined as time from treatment initiation (nivolumab or chemotherapy) to progression or death, whichever occurred first. Overall survival (OS) was defined as time from treatment initiation to death from any cause. DCR was defined as complete plus partial response plus stable disease, and ORR as complete plus partial response

### **2.4. Statistical analysis**

Patient and tumor characteristics were compared between patients with high-TILs versus non-high-TILs using a Wilcoxon Mann Withney or Chi<sup>2</sup> / Fisher tests. To assess the association of TIL with survival endpoints, we used a multivariate Cox model including lung cancer prognostic factors (age,

line of treatment, number of metastatic sites, performance status [PS], histology). Correlation between variables was verified before construction of the multivariate model, to deal with potential co-linearity. The median follow-up was calculated with the reverse Kaplan-Meier method. For the immunotherapy-cohort, the association between patient characteristics and response was assessed with a univariate logistic regression model. All analyses were performed using R version 2.15.2 and SAS 9.4 software. All tests and analyses were performed at the two-side 5% alpha level.

### 3. RESULTS

#### 3.1. Immunotherapy cohort

Baseline characteristics of the 221 patients are summarized in **Table 1**. PD-L1 was positive in 38 (48%), negative in 41 (52%) and not reported in 142 patients. With a median follow-up of 11.0 months [95% CI 10.0 to 23.0], median OS was 11.0 months [95% CI, 7.0 to 13.2] and median PFS was 3.1 months [95% CI, 2.5 to 4.9]. ORR and DCR were 22% and 57% respectively.

Mean TIL expression in stroma was 10% (0-60). High-TIL was observed in 28% (49/175) of patients, and was non-assessable in 46. Clinical characteristics according to TIL expression are summarized in **Table 2**. In multivariate analysis, high-TIL was independently associated with better OS [hazard ratio HR 0.48; 95%CI 0.28-0.81];  $P=.006$  and PFS [HR 0.40; 95%CI 0.25-0.64];  $P<.0001$  (**Table 3**).

In the high-TIL group, median OS was not reached in the high-TIL group vs. 8.4 months [95%CI 5.0-11.6] in the low-TIL group ( $P=.007$ ) (**Figure 2**). Median PFS was 13.0 months. [95%CI 5.0-not reached] vs. 2.2 months [95%CI 1.7-3.0] in low-TIL group ( $P<.0001$ ) (**Figure 2**). High-TIL was associated with both ORR ( $P<.0001$ ) and DCR ( $P<.0001$ ) (**Table 4**).

In the subpopulation with PD-L1 status available (N=79), high-TIL and PD-L1 expression were not associated ( $r=0.22$ ,  $P=.06$ ) (**Figure 3**).

#### 3.2. Chemotherapy cohort

Baseline characteristics of the 189 patients are summarized in **Table 1**. Median follow-up was 75 months [95%CI 42.4-82.9]. High-TIL was observed in 37% (70/189). Clinical characteristics according to TIL expression are summarized in **Table 2**. In the multivariate analysis, no significant association was observed between TIL and PFS [HR 1.10; 95%CI 0.79-1.49],  $P=0.61$  or OS [HR 1.23; 95%CI 0.89-1.71],  $P=0.21$ .

Median PFS and OS were 5.7 months [ 95%CI 4.9-6.7] and 11.7 months [ 95%CI 9.3-13.0] respectively. In the high-TIL group, median OS was 11.6 months [ 95%CI 8.2-13.4] vs. 11.8 months [ 95%CI 8.8-14.0] in low-TIL group ( $P=0.268$ ) (**Figure 2**). In the high-TIL group, median PFS was 5.3 months [ 95 CI% 4.2 to 7.0] vs. 5.9 months [ 95%CI 4.9-7.4] in low-TIL group ( $P=0.74$ ) (**Figure 2**). PD-L1 status was not available for this population and cannot be evaluated retrospectively because of the unavailability of tumor samples in most cases.

#### 4. DISCUSSION

In this study, we reported for the first time an association of morphologically assessed high-TIL density with favorable immunotherapy outcomes in advanced NSCLC patients [HR for PFS 0.40; HR for OS 0.48]. However, no association was observed in CC, suggesting a potential role for TIL in predicting ICI benefit. Unlike in breast cancer, very few studies have evaluated the prognostic impact of TIL density in H&E sections. Our evaluation was based on the stromal compartment, since morphological assessment of intraepithelial lymphocytes is more variable and less reproducible. Based on a  $\geq 10\%$  cut-off, we found high-TIL in 28% of cases in the IC and in 37% of cases in the CC, consistent with previous reports <sup>7</sup>. In most NSCLC studies, TIL evaluation was performed using IHC staining of T cells, but no standardized methodology has been defined. The morphological TIL assessment on H&E slides offers a straightforward option for routine use, with a methodology for detection of lymphocytes similar between pathology laboratories, and standardized pathological assessments proposed<sup>7</sup>. In lung cancer, a few studies have assessed TIL, mainly assessed by IHC staining. However, to our knowledge, no studies have evaluated the prognostic value of morphological assessment of TIL in NSCLC, and the clinical relevance for immunotherapy. In early stages, high CD8+ cells in stroma (58% of cases) and high CD4+ cells (74%) were significantly associated with disease-specific survival <sup>8</sup>, consistent with other studies in early disease <sup>9,10</sup>. In a meta-analysis of 29 studies, high CD8+ in stromal, heterogeneously defined (ranging from  $> 50\%$  to  $> 383/\text{mm}^2$ ), were associated with better OS [HR=0.76, 95% CI 0.62-0.93] in all-stage lung cancer patients, although a minority (6.3%) had stage IV <sup>6</sup>. Interestingly, high-TIL were not associated with chemotherapy response, in line with our findings. Lymphocytes represent the predominant cytotoxic population in the adaptive response against cancer-specific antigens <sup>6</sup>. TIL reflect a broad evaluation of the level of the adaptive immune response against

tumor cells, reported previously as the “hot” or “cold” immune phenotype. It includes a heterogeneous subpopulation with different functions, including CD8+ cytotoxic T cells, CD4+ helper cells, CD45RO+ memory T cells, etc. We previously observed a degree of correlation between TIL density and CD3+CD8+ cell density, which may explain why TIL can correlate with better responses to ICIs (Corgnac S *et al*, submitted).

However, our study has several limitations. As a retrospective study, some clinical and pathological data are missing; e.g. PD-L1 status which was not determined at the time of treatment for most patients (as it was not required for prescribing nivolumab). The combination of PD-L1 and TIL assessment may help enhancing the prediction of ICI benefit. Secondly, archival tumor specimens were used with most patients in the IC having received chemotherapy, whereas evaluation of pretreatment TIL in a recent specimen may be a more clinically-relevant approach. Finally, the morphological evaluation of TIL includes all T cell subpopulations (as well as some B cells), which potentially have an opposite impact on prognosis and benefit from ICIs<sup>11</sup>. Moreover, other immune cell subpopulations modulating TIL activity have not been explored (e.g. neutrophils, macrophages, myeloid derived suppressor cells, etc.). Despite all these, we believe our work demonstrated the clinical relevance of high-TIL as a simple assessment, accessible worldwide, without cost and tissue consuming, and strongly associated with immunotherapy outcomes.

## **5. CONCLUSION**

In conclusion, high-TIL density (>10%) by morphological assessment on H&E slides, is associated with immunotherapy outcomes in advanced NSCLC, but not with chemotherapy. TIL morphological evaluation is a simple and accessible tool that merits validation in larger prospective studies.

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## FIGURE AND TABLES LEGENDS

**Figure 1.** Study Flow Chart.

**Table 1.** Baseline Characteristics of the immunotherapy cohort (IC) and chemotherapy cohort (CC).

**Table 2.** Baseline Characteristics of the immunotherapy cohort (IC) and chemotherapy cohort (CC), according to the TIL.

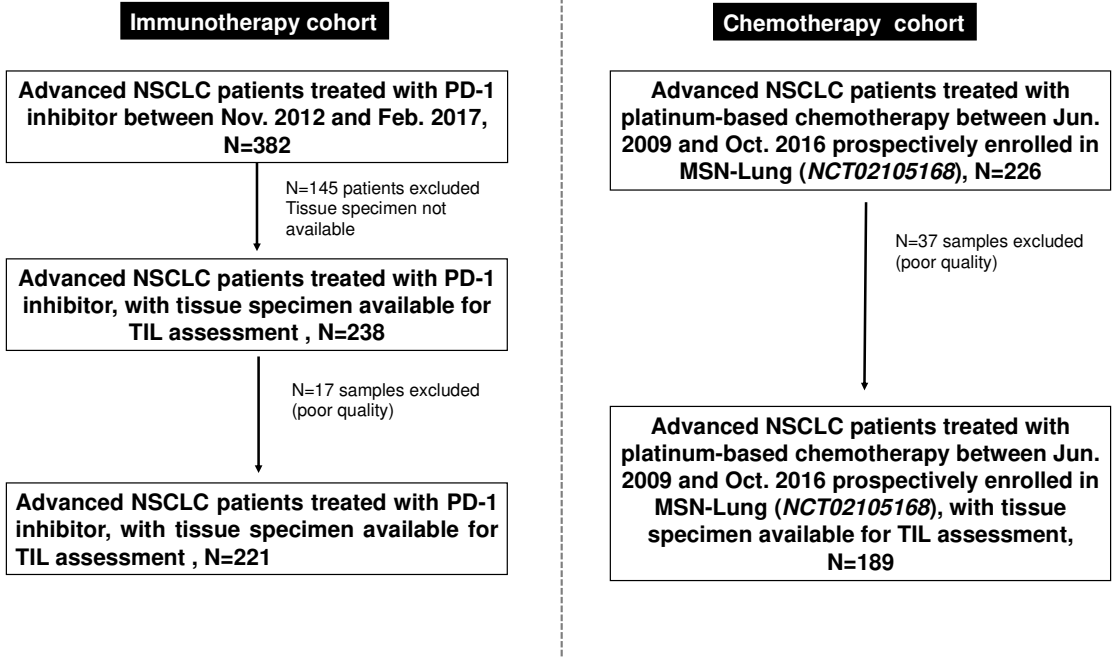
**Figure 2.** Overall Survival (OS) and Progression-Free Survival (PFS) according to TIL, in the immunotherapy cohort (IC) and in the chemotherapy cohort (CC).

**Table 3.** Multivariate analysis on overall survival (OS), in the immunotherapy cohort (IC) and in the chemotherapy cohort (CC).

**Table 4.** Univariate analysis, in the immunotherapy cohort (IC) for ORR and DCR.

**Figure 3.** Progression-Free Survival (PFS) and Overall Survival (OS) according to TIL and PDL1, in the immunotherapy cohort (IC) and in the chemotherapy cohort (CC).

Figure1. Study Flow Chart.



**Table1.** Baseline Characteristics of the immunotherapy cohort (IC) and chemotherapy cohort (CC)

		IC (N=221)		CC (N=189)	
<b>Age (years)</b>	Median (Q1;Q3)	62.3 (57 ;68)		63 (57 ; 69)	
	≤ 70	175	79%	147	77.8%
	>70	46	21%	42	22.2%
<b>Gender</b>	Male	142	64%	131	69%
	Female	79	36%	58	31%
<b>Smoking status</b>	No smoker	34	15.4	21	11%
	Ex smoker	105	47.5	125	66%
	Current smoker	77	34.8%	43	23%
	Unknown	5	2.3	0	0
<b>Histology</b>	Non-Squamous	162	73%	170	90%
	Squamous	59	27%	19	10%
<b>Number of chemotherapy lines</b>	≤ 2	125	57%	123	65%
	> 2	70	31.82%	65	35%
<b>Molecular status</b>	<i>EGFR</i> mutation	9	4%	3	3%
	<i>ALK</i> fusion	2	1%	2	1%
	<i>BRAF</i> mutation	4	2%	-	-
	<i>ROS1</i> fusion	1	< 1%	-	-
	<i>KRAS</i> mutation	48	23%	25	28%
<b>ECOG Performance Status</b>	0-1	161	77%	162	86%
	≥ 2	48	23%	27	14%

<sup>a)</sup> Line of treatment: lines of immunotherapy for immunotherapy cohort; lines of chemotherapy for the chemotherapy cohort



**Table2.** Baseline Characteristics of the immunotherapy cohort (IC) and chemotherapy cohort (CC), according to TIL density.

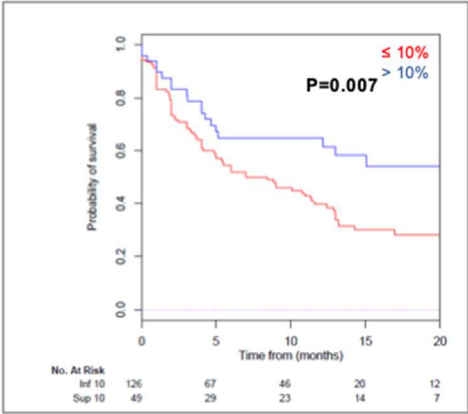
Characteristics	IC (N=221)			CC (N=189)		
	TIL ≤10% (N=126 <sup>a</sup> )	High-TIL >10% (N=49 <sup>a</sup> )	P value	TIL ≤10% (N=119)	High-TIL >10% (N=70)	P value
<b>Age – years</b> > 70 years- no (%)	29 (23) <sup>a</sup>	6 (12) <sup>a</sup>	0.11	22 (19)	20 (28)	0.11
<b>Sex - N (%)</b> Male Female	79 (63) 47 (37)	28 (57) 21 (43)	0.498	79 (66) 40 (34)	52(74) 18 (26)	0.26
<b>PS ECOG– no (%)</b> 0-1 ≥ 2 Unknown	92 (73) 30 (23.8) 4 (3.2)	35 (71.4) 9 (18.4) 5 (10.2)	0.83	103 (87) 16 (13) 0	59 (84) 11 (16) 0	0.67
<b>Smoking history–no (%)</b> Current Former Never Unknown	40 (31.8) 59 (46.8) 24 (19) 3 (2.4)	19 (38.8) 26 (53) 2 (4.1) 2 (4.1)	<b>0.036</b>	25 (21) 78 (66) 16 (13) 0	43 (22.8) 125 (66.1) 21 (11.1) 0	0.36
<b>Histology – no (%)</b> Squamous No squamous	40 (32) 86 (68)	12 (24) 37 (76)	0.35	8 (7) 111 (93)	11 (16) 59 (84)	<b>0.047</b>
<b>Molecular status – no (%)</b> EGFR mutation ALK fusion BRAF mutation ROS1 fusion KRAS mutation	7 (7) 1 (<1) 1 (<1) 1 (<1) 22 (21)	0 0 0 0 16 (24)	0.97	3 (5) 2 (2) - - 15 (26)	- - - - 10 (31)	NS
<b>Treatment line <sup>(a)</sup>– no (%)</b> ≤ 2 <sup>nd</sup> line > 2 <sup>nd</sup> line Unknown	63 (50) 63 (50) 0	32 (65.3) 17 (34.7) 0	0.068	81 (68.1) 38 (31.9) 0	42 (60) 27 (38.6) 1 (1.4)	0.32
<b>Metastatic sites - no (%)</b> ≤ 2 sites > 2 sites	75 (60) 50 (40)	37 (75.5) 12 (24.5)	0.055	58(49) 61(51)	32(46) 38(54)	0.69

a) Missing data: Unknown TIL=46(20.8%)

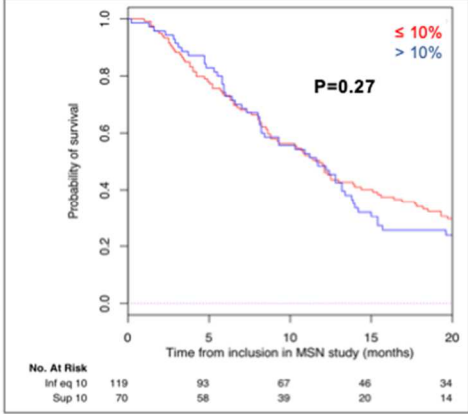
Line of treatment: line of immunotherapy for immunotherapy cohort; line of chemotherapy for the chemotherapy cohort

**Figure 2.** Overall survival (OS) and progression-free survival (PFS) according to tumor infiltrating lymphocytes (TIL) density, in the immunotherapy cohort (IC) and in the chemotherapy cohort (CC)

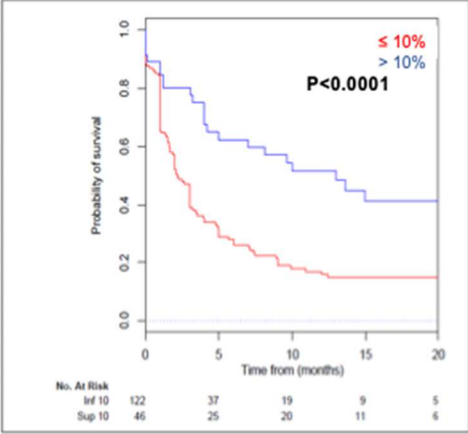
**A. OS in the immunotherapy cohort according to >10% TIL**



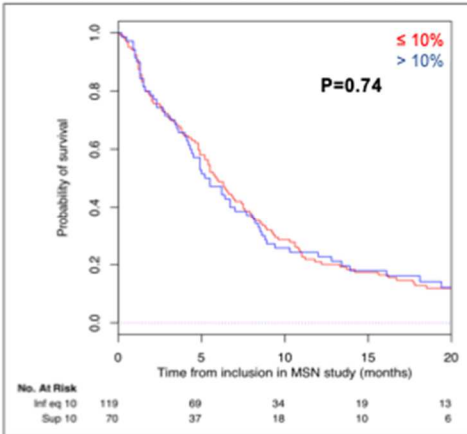
**B. OS in the chemotherapy cohort according to > 10% TIL**



**C. PFS in the immunotherapy cohort according to >10% TIL**



**D. PFS in the chemotherapy cohort according to > 10% TIL**



**Table 3.** Multivariate analysis on overall survival (OS), in the immunotherapy cohort (IC) and in the chemotherapy cohort (CC).

	<b>Immunotherapy cohort (n=221)</b>		<b>Chemotherapy cohort (n=188)</b>	
	Hazard ratio (HR) 95% confidence interval (CI)	P-value	(HR) 95% confidence interval (CI)	P-value
<b>TIL</b> > 10%	0.48 (0.28-0.81)	<b>0.006</b>	1.23 (0.89-1.71)	0.21
<b>Age</b> ≥ 70 years	0.85 (0.50-1.46)	0.57	1.13 (0.76-1.66)	0.55
<b>Line of treatment</b> <sup>(a)</sup> ≥ 2 lines	0.69 (0.44-1.09)	0.11	0.84 (0.58-1.22)	0.47
<b>Number metastatic sites</b> ≥ 2	1.40 (0.88-2.20)	0.16	1.58 (1.12-2.22)	<b>0.01</b>
<b>Performance status</b> ≥ 2	2.75 (1.72-4.36)	<b>&lt;0.0001</b>	1.96 (1.26-3.00)	<b>&lt;0.01</b>
<b>Histology</b> Squamous	1.13 (0.70-1.80)	0.62	1.05 (0.62-1.77)	0.85

<sup>(a)</sup>Line of treatment: line of immunotherapy for immunotherapy cohort; line of chemotherapy for the chemotherapy cohort.

**Table 4.** Univariate analysis, in the immunotherapy cohort (IC) for ORR and DCR

	<b>ORR+, Immuno cohort (n=221)</b>		<b>DCR*, Immuno cohort (n=221)</b>	
	<b>Hazard ratio (HR)</b> <b>95% confidence interval (CI)</b>	<b>P-value</b>	<b>(HR) 95% confidence interval (CI)</b>	<b>P-value</b>
<b>TIL</b> > 10%	4.61 (2.00- 10.62)	< <b>0.0001</b>	6.12 (2.5-14.98)	< <b>0.0001</b>
<b>Age</b> ≥ 70years	1.58 (0.71- 3.54)	0.26	1.46 (0.71- 3.03)	0.31
<b>Line of treatment<sup>(a)</sup></b> ≥ 2 lines	0.41 (0.19-0.86)	0.019	0.68 (0.38-1.21)	0.19
<b>Number metastatic sites</b> ≥ 2	0.72 (0.33- 1.61)	0.42	0.35 (0.18-0.66)	0.001
<b>Histology</b> Squamous	1.07 (0.49-2.34)	0.86	1.28 (0.66- 2.48)	0.47
<b>PS</b> ≥ 2	0.32 (0.09-1.14)	0.078	0.41 (0.14- 1.24)	0.12
<b>Smokers status</b> Former	2.24 (0.96- 5.23)	0.064	1.00 (0.52- 1.93)	0.98

+: ORR: objective response rate; \*DCR: disease control rate; <sup>(a)</sup>Line of treatment: lines of immunotherapy for immunotherapy cohort; lines of chemotherapy for the chemotherapy cohort.

**Figure 3.** Progression-Free Survival (PFS) and Overall Survival (OS) according to TIL density and PD-L1 expression, in the immunotherapy cohort (IC).

