Supplemental material

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J Immunother Cancer

1   **Supplemental materials**

2   **Materials and Methods**

3   **Patients**

4   The inclusion criteria for treatment naïve cohort were operable lung cancer patients who underwent

5   upfront surgery with pathology-confirmed stage II-IIIA NSCLC according to the eighth edition of the

6   TNM classification for lung cancer. The inclusion criteria for chemoimmunotherapy and

7   chemotherapy cohort were as follows: (1) patients with NSCLC (II-IIIA stage) confirmed by

8   bronchoscopy biopsy or computed tomography (CT) guided puncture biopsy; (2) the preoperative

9   staging done with contrast-enhanced computed tomography (CT) or positron emission tomography

10   (PET); (3) neoadjuvant platinum-based chemotherapy combined with anti-PD-1 (Nivolumab,

11   Camrelizumab, or Tislelizumab) or neoadjuvant platinum-based chemotherapy alone administered

12   for at least 2 cycles before surgery; (4) formalin-fixed, paraffin-embedded (FFPE) surgical tumor

13   tissue samples available. Chemotherapy regimens were selected according to the National

14   Comprehensive Cancer Network (NCCN) guidelines. For non-squamous NSCLC, cisplatin or

15   carboplatin plus Pemetrexed were administered; and for squamous NSCLC, cisplatin or carboplatin

16   plus paclitaxel were administered. Cisplatin was preferred, and carboplatin used for the patients with

17   comorbidities or not able to tolerate cisplatin. 121 cancer tissue samples were obtained during

18   surgical resection. In the neoadjuvant chemoimmunotherapy group, 7 biopsies were collected by

19   bronchoscopy or CT-guided percutaneous lung puncture within 3 weeks before neoadjuvant therapy.

20   All samples were immediately formalin-fixed and paraffin-embedded (FFPE) after tissue collection.

21   **Multiplex immunohistochemistry (mIHC)**

22   Staining was conducted using the Akoya OPAL Polaris 7-Color Automation IHC kit

23   (NEL871001KT). FFPE tissue slides were first deparaffinized in a BOND RX system (Leica

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24 Biosystems) and then incubated sequentially with primary antibodies targeting CD163 (Abcam,

25 ab182422, 1:500), CD68 (Abcam, ab213363, 1:1000), PD-1 (CST, D4W2J, 1:200), PD-L1 (CST,

26 E1L3N, 1:400), CD3 (Dako, A0452, 1:1), CD4 (Abcam, ab133616, 1:100), CD8 (Abcam, ab178089,

27 1:200), CD56 (Abcam, ab75813, 1:1000), CD20 (Dako, L26, IR604, 1:1), FOXP3 (Abcam, ab20034,

28 1:100) and pan-CK (Abcam, ab7753, 1:100). Nuclei were stained with DAPI. Multiplex stained

29 slides were scanned using a Vectra Polaris Quantitative Pathology Imaging System (Akoya

30 Biosciences) at 20 nm wavelength intervals from 440 nm to 780 nm with a fixed exposure time and

31 an absolute magnification of $\times$ 200. All scans for each slide were then superimposed to obtain a

32 single image. Multilayer images were imported to inForm v.2.4.8 (Akoya Biosciences) for

33 quantitative image analysis. Tumor parenchyma and stroma were differentiated by Pan-CK staining.

34 The quantities of various cell populations were expressed as the number of stained cells per square

35 millimeter and the percentage of positively stained cells among all nucleated cells.

Supplemental table 1. Treatment regimens for different treatment groups

Cohort	Histology	Treatment options	Patients number
Neoadjuvant chemoimmunotherapy	Non-squamous NSCLC	Nivolumab 360 mg + Cisplatin 75 mg/m^2 or Carboplatin AUC 5 + Pemetrexed 500 mg/m^2	10
		Camrelizumab 200 mg + Cisplatin 75 mg/m^2 or Carboplatin AUC 5 + Pemetrexed 500 mg/m^2	3
		Tislelizumab 200 mg + Cisplatin 75 mg/m^2 or Carboplatin AUC 5 + Pemetrexed 500 mg/m^2	6
	Squamous NSCLC	Nivolumab 360 mg + Cisplatin 75 mg/m^2 or Carboplatin AUC 5 + Paclitaxel-albumin 135 mg/m^2	12

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		Camrelizumab 200 mg + Cisplatin 75 mg/m^2 or Carboplatin AUC 5 + Paclitaxel-albumin 135 mg/m^2	3
		Tislelizumab 200 mg + Cisplatin 75 mg/m^2 or Carboplatin AUC 5 + Paclitaxel-albumin 135 mg/m^2	6
Neoadjuvant chemotherapy	Non-squamous NSCLC	Cisplatin 75 mg/m^2 or Carboplatin AUC 5 + Pemetrexed 500 mg/m^2	16
	Squamous NSCLC	Cisplatin 75 mg/m^2 or Carboplatin AUC 5 + Paclitaxel-albumin 135 mg/m^2	25

Supplemental table 2. Univariate analysis of MPR for neoadjuvant chemoimmunotherapy and neoadjuvant chemotherapy group

Characteristics		Neoadjuvant chemoimmunotherapy			Neoadjuvant chemotherapy		
		Non-MPR	MPR	P-value Odds ratio (95% CI)	Non-MPR	MPR	P-value Odds ratio (95% CI)
Gender	Male	12	13	0.254 2.167	28	4	0.797 1.034
	Female	10	5	(0.573-8.190)	6	3	(0.102-10.527)
Age	< 63	13	6	0.109 0.346	18	5	0.377 2.222
	≥ 63	9	12	(0.095-1.267)	16	2	(0.377-13.082)
Smoking history	Smoker or ex-smoker	15	15	0.278 2.333 (0.505-10.778)	27	6	0.703 1.556 (0.160-15.123)

	Never smoker	7	3		7	1	
Histology				0.017			0.512
	Squamous cell carcinomas	8	13	0.621 0.542 (0.048-6.144)	21	4	0.274 0.190 (0.010-3.716)
	Adenocarcinoma	13	2	0.032 0.051 (0.003-0.770)	12	2	0.265 0.167 (0.007-3.890)
	Large cell carcinoma	1	3	1	1	1	1
N stage	N0	14	15	0.174 2.857 (0.629-12.981)	18	6	0.140 5.333 (0.578-49.181)
	N1-2	8	3		16	1	
Neoadjuvant therapy number of cycles	2	4	12	0.003 9.000 (2.088-38.787)	24	1	0.020 0.069 (0.007-0.654)
	>2	18	6		10	6	
TLS maturation	Low-maturation	9	1	0.027 0.085 (0.010-0.758）	27	1	0.007 0.043 (0.004-0.420)
	High-maturation	13	17		7	6	

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				P-value / OR (95% CI)				P-value / OR (95% CI)
TLS abundance				0.058				0.989
	Score 0	5	1	1	20	0		1
	Score 1	5	3	0.035 0.073 (0.006-0.829)	8	0	0.998	- -
	Score 2	8	3	0.103 0.218 (0.035-1.364)	2	3	0.999	- -
	Score 3	4	11	0.026 0.136 (0.024-0.786)	4	4	0.725 1.500 (0.156-14.420)

Supplemental table 3. The correlation between TLS maturation and clinicopathological features

Characteristics		Neoadjuvant chemoimmunotherapy			Neoadjuvant chemotherapy			Treatment naïve		
		Low-maturation TLS	High-maturation TLS	P-value	Low-maturation TLS	High-maturation TLS	P-value	Low-maturation TLS	High-maturation TLS	P-value
Gender	Male	5	20	0.35	21	11	0.93	8	14	0.87
	Female	5	10		7	2		7	11	
Smoking history	Smoker or	7	23	0.67	22	11	0.65	10	16	0.86

	ex-smoker									
	Never smoker	3	7		6	2		5	9	
Histology	Squamous cell carcinomas	4	17	0.63	18	7	0.10	5	9	0.17
	Adenocarcinoma	5	10		10	4		8	16	
	Large cell carcinoma	1	3	1	0	2	1	2	0	
N stage	N0	5	24	0.07	16	8	0.79	10	18	0.72
	N1-2	5	6		12	5		5	7	
Neoadjuvant therapy number of cycles	2	4	12	0.65	18	7	0.52	-	-	
	>2	6	18		10	6		-	-	

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