

Dynamic changes of peripheral NK cells predict outcome in patients with PD-L1 positive Non-Small-Cell Lung Cancer undergoing immune checkpoint inhibitors as second-line therapy

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Supplementary Table S1. Distribution of clinical and pathological characteristics at baseline across disease outcome subgroups

Variable	DCB N=23 (100%)	NCB N=18 (100%)	<i>P</i> value
Age			
- median (range)	68 (39-77)	67 (49-76)	0.87
Sex			
- female (n=17)	10 (43%)	7 (39%)	0.76
- male (n=24)	13 (57%)	11 (41%)	
ECOG Performance Status			
- 0 (n=21)	13 (57%)	8 (44%)	0.44
- 1 (n=20)	10 (43%)	10 (56%)	
Histology			
- adenocarcinoma (n=27)	14 (61%)	13 (72%)	0.44
- squamous-cell carcinoma (n=14)	9 (9%)	5 (28%)	
Stage			
- IIIB or IIIC (n=3)	2 (10%)	1 (6%)	0.66
- IV (n=38)	21 (90%)	17 (94%)	
Smoking habits			
- ever smoker (n=34)	18 (78%)	16 (89%)	0.36
- never smoker (n=7)	5 (22%)	2 (11%)	
Number of metastatic sites			
- 1 site (n=15)	12 (52%)	3 (17%)	0.019
- ≥ 2 sites (n=26)	11 (48%)	15 (83%)	
Weight loss			
- <10% (n=22)	16 (69%)	6 (33%)	0.021
- $\geq 10\%$ (n=19)	7 (31%)	12 (67%)	
Liver metastases			
- no (n=32)	22 (95%)	10 (55%)	0.002
- yes (n=9)	1 (5%)	8 (45%)	
Bone metastases			
- no (n=27)	16 (69%)	11 (61%)	0.57
- yes (n=14)	7 (31%)	7 (39%)	

Brain metastases			
- no (n=31)	18 (78%)	13 (72%)	0.65
- yes (n=10)	5 (22%)	5 (28%)	
PD-L1 TPS			
- <1% (n=15)	4 (17%)	11 (61%)	0.004
- ≥1% (n=26)	19 (83%)	7 (39%)	
First line chemotherapy			0.67
- carboplatin-based (n=22)	13 (57%)	9 (50%)	
- cisplatin-based (n=19)	10 (43%)	9 (50%)	
First line chemotherapy			0.70
- antimetabolite-based ^a (n=26)	14 (61%)	12 (67%)	
- taxane-based ^b (n=15)	9 (39%)	6 (33%)	
Best response to previous chemotherapy			
- SD or CR/PR (n=25)	14 (61%)	11 (61%)	0.98
- PD (n=16)	9 (9%)	7 (39%)	
Time from completion of first line chemotherapy			
- <3 months (n=20)	12 (52%)	8 (44%)	0.62
- ≥3 months (n=21)	11 (48%)	10 (56%)	
Concomitant medications ^c			0.94
- corticosteroids ^d (n=20)	10 (43%)	10 (56%)	
- proton pump inhibitors (n=24)	12 (52%)	12 (67%)	
- antibiotics (n=8)	5 (22%)	3 (17%)	
- statins (n=14)	8 (35%)	6 (33%)	
- metformin (n=10)	6 (26%)	4 (22%)	
ICI second line therapy			0.89
- nivolumab (n=22)	13 (57%)	9 (50%)	
- pembrolizumab (n=11)	6 (26%)	5 (28%)	
- atezolizumab (n=8)	4 (17%)	4 (22%)	

Abbreviations: DCB, durable control benefit; NCB, no control benefit; ECOG PS, Eastern Cooperative Oncology Group Performance Status; CR/PR, complete response/partial response; SD, stable disease; PD, progressive disease; PD-L1 TPS, programmed cell death-ligand 1 tumor proportion score; ICI, immune checkpoint inhibitor.

^a antimetabolite-based chemotherapy included regimens containing gemcitabine or pemetrexed; ^b taxane-based chemotherapy included regimens containing paclitaxel or docetaxel; ^c concomitant medications indicate the intake of any of the following drugs in the 14 days preceding the start of ICI treatment; ^c corticosteroid therapy indicates <10 mg prednisone equivalent daily.

Supplementary Table S2. Multivariate analysis of peripheral lymphocyte counts by predefined clinical variables at time point 1.

Variable	T helper cell count (log)		T cytotoxic cell count (log)		B cell count (log)		NK cell count (log)	
	Beta (95% CI)	P value	Beta (95% CI)	P value	Beta (95% CI)	P value	Beta (95% CI)	P value
Sex								
- male vs female	-0.04 (-0.09 to 0.01)	0.12	0.04 (-0.05 to 0.13)	0.41	0.02 (-0.06 to 0.12)	0.57	-0.01 (-0.09 to 0.09)	0.98
Age (years)								
- ≥65 vs <65	-0.10 (-0.23 to 0.01)	0.83	-0.05 (-0.27 to 0.16)	0.64	-0.07 (-0.28 to 0.15)	0.53	0.05 (-0.15 to 0.26)	0.61
ECOG PS								
- 1 vs 0	-0.01 (-0.06 to 0.03)	0.57	0.01 (-0.06 to 0.10)	0.65	0.06 (-0.01 to 0.15)	0.12	-0.04 (-0.13 to 0.03)	0.24
Histology								
- squamous-cell vs adenocarcinoma	0.13 (0.04 to 0.22)	0.005	-0.10 (-0.06 to 0.27)	0.23	-0.01 (-0.17 to 0.16)	0.96	-0.06 (-0.23 to 0.09)	0.43
Stage								
- IV vs IIIB or C	0.10 (-0.01 to 0.20)	0.05	0.04 (-0.14 to 0.23)	0.62	-0.08 (-0.26 to 0.10)	0.39	-0.14 (-0.32 to 0.03)	0.10
Smoking habits								
- ever vs never smoker	0.01 (-0.04 to 0.07)	0.62	0.22 (0.10 to 0.33)	<0.001	0.04 (-0.06 to 0.16)	0.42	-0.03 (-0.14 to 0.07)	0.57
Number of metastatic sites								
- ≥2 vs 1 site	-0.03 (-0.09 to 0.02)	0.21	-0.08 (-0.19 to 0.02)	0.14	-0.08 (-0.18 to 0.02)	0.14	-0.22 (-0.32 to -0.11)	<0.001
Weight loss								
- ≥10% vs <10%	-0.01 (-0.05 to 0.04)	0.76	0.05 (-0.03 to 0.14)	0.20	0.03 (-0.05 to 0.12)	0.45	0.01 (-0.07 to 0.09)	0.88
Liver metastases								
- yes vs no	0.04 (-0.01 to 0.09)	0.11	0.07 (-0.02 to 0.17)	0.11	0.06 (-0.03 to 0.15)	0.22	0.03 (-0.05 to 0.13)	0.41
Bone metastases								
- yes vs no	0.03 (-0.02 to 0.08)	0.23	0.08 (-0.01 to 0.18)	0.07	0.08 (-0.02 to 0.21)	0.017	0.06 (-0.33 to 0.15)	0.20
Brain metastases								
- yes vs no	-0.03 (-0.10 to 0.03)	0.27	-0.09 (-0.22 to 0.02)	0.11	0.02 (-0.09 to 0.15)	0.64	0.03 (-0.08 to 0.15)	0.55
PD-L1 TPS								

- ≥1% vs <1%	0.01 (-0.05 to 0.05)	0.98	-0.02 (-0.12 to 0.07)	0.61	0.04 (-0.05 to 0.14)	0.33	0.15 (0.05 to 0.24)	0.002
First line chemotherapy - carboplatin vs cisplatin-based	-0.03 (-0.15 to 0.08)	0.59	0.01 (-0.19 to 0.22)	0.89	-0.07 (-0.28 to 0.13)	0.49	0.09 (-0.10 to 0.30)	0.34
First line chemotherapy - antimetabolite ^a vs taxane ^b	0.04 (-0.01 to 0.10)	0.13	-0.02 (-0.13 to 0.08)	0.69	-0.10 (-0.21 to 0.01)	0.07	-0.12 (-0.23 to -0.01)	0.022
Best response to previous chemotherapy - SD/CR/PR vs PD	-0.02 (-0.06 to 0.02)	0.29	-0.01 (-0.08 to 0.07)	0.95	-0.03 (-0.11 to 0.04)	0.44	-0.12 (-0.09 to 0.06)	0.76
Time from completion of previous chemotherapy - ≥3 months vs <3 months	-0.01 (-0.05 to 0.03)	0.60	-0.02 (-0.10 to 0.05)	0.54	0.07 (-0.01 to 0.15)	0.06	0.07 (-0.01 to 0.15)	0.05
Corticosteroids ^c - yes vs no	0.09 (0.03 to 0.16)	0.004	0.15 (0.03 to 0.27)	0.010	-0.05 (-0.17 to 0.06)	0.40	-0.12 (-0.23 to -0.01)	0.038
Proton pump inhibitors - yes vs no	-0.04 (-0.10 to 0.01)	0.12	-0.06 (-0.17 to 0.04)	0.26	0.04 (-0.06 to 0.15)	0.43	0.12 (0.01 to 0.22)	0.022
Antibiotics - yes vs no	-0.04 (-0.10 to 0.01)	0.15	-0.08 (-0.18 to 0.02)	0.13	0.26 (0.16 to 0.36)	<0.001	-0.04 (-0.14 to 0.05)	0.41
Statins - yes vs no	-0.01 (-0.06 to 0.04)	0.82	-0.12 (-0.22 to -0.02)	0.015	-0.09 (-0.18 to 0.01)	0.07	0.02 (-0.06 to 0.12)	0.57
Metformin - yes vs no	-0.07 (-0.14 to -0.01)	0.025	-0.01 (-0.13 to 0.10)	0.79	0.10 (-0.02 to 0.22)	0.10	0.02 (-0.09 to 0.13)	0.72

Abbreviations: log, logarithmic values; ECOG PS, Eastern Cooperative Oncology Group Performance Status; CR/PR, complete response/partial response; SD, stable disease; PD, progressive disease; PD-L1 TPS, programmed cell death-ligand 1 tumor proportion score.

^a antimetabolite-based chemotherapy included regimens containing gemcitabine or pemetrexed; ^b taxane-based chemotherapy included regimens containing paclitaxel or docetaxel; ^c corticosteroids indicate <10 mg prednisone equivalent daily. Time point 1 denotes baseline assessment.

Supplementary Table S3. Multivariate analysis of peripheral lymphocyte counts by predefined clinical variables at time point 2.

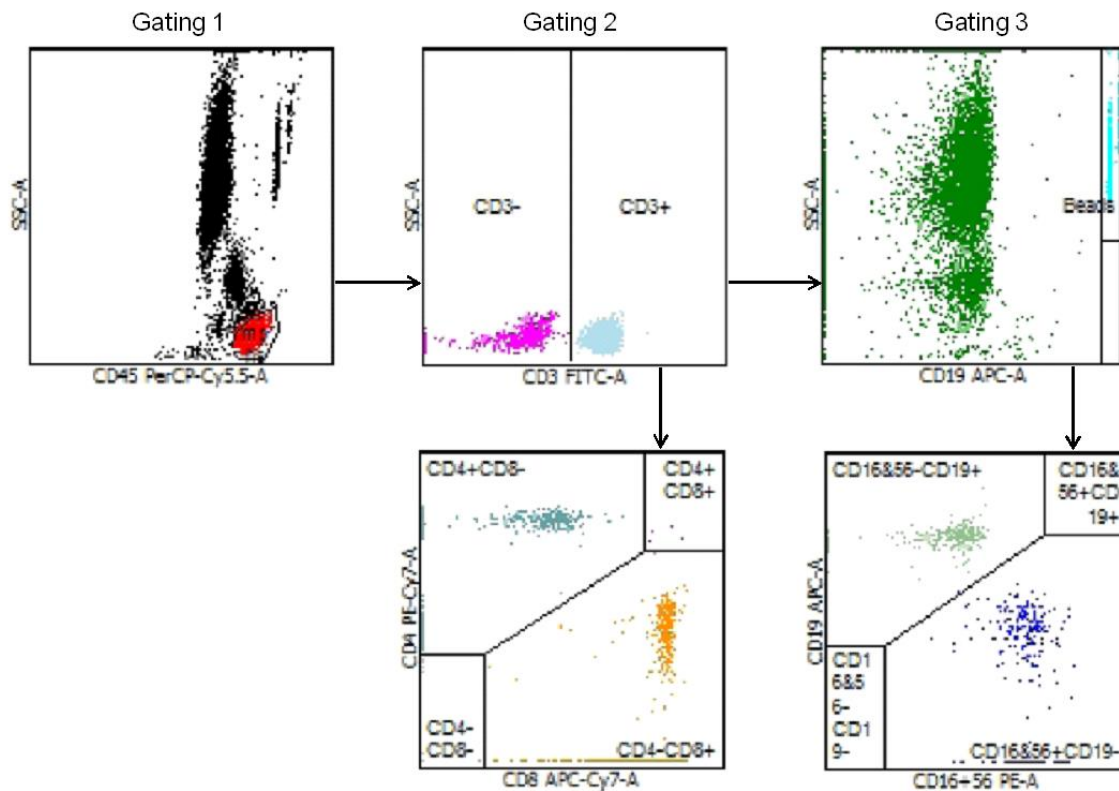
Variable	T helper cell count (log)		T cytotoxic cell count (log)		B cell count (log)		NK cell count (log)	
	Beta (95% CI)	P value	Beta (95% CI)	P value	Beta (95% CI)	P value	Beta (95% CI)	P value
Sex								
- male vs female	0.02 (-0.01 to 0.06)	0.22	0.03 (-0.06 to 0.13)	0.51	0.03 (-0.05 to 0.11)	0.50	-0.01 (-0.08 to 0.05)	0.72
Age (years)								
- ≥65 vs <65	-0.05 (-0.14 to 0.03)	0.23	0.03 (-0.19 to 0.25)	0.79	0.12 (-0.07 to 0.32)	0.23	-0.07 (-0.22 to 0.08)	0.35
ECOG PS								
- 1 vs 0	-0.01 (-0.05 to 0.02)	0.38	0.01 (-0.07 to 0.10)	0.76	-0.09 (-0.17 to -0.01)	0.019	0.06 (0.01 to 0.129)	0.035
Histology								
- squamous-cell vs adenocarcinoma	0.04 (-0.02 to 0.11)	0.22	0.13 (-0.04 to 0.30)	0.14	0.02 (-0.13 to 0.17)	0.78	0.06 (-0.05 to 0.18)	0.27
Stage								
- IV vs IIIB or C	0.04 (-0.03 to 0.12)	0.26	0.06 (-0.12 to 0.25)	0.52	0.09 (-0.08 to 0.26)	0.30	-0.01 (-0.14 to 0.11)	0.79
Smoking habits								
- ever vs never smoker	0.04 (-0.01 to 0.08)	0.09	0.08 (-0.03 to 0.20)	0.15	-0.01 (-0.12 to 0.08)	0.74	-0.03 (-0.11 to 0.04)	0.42
Number of metastatic sites								
- ≥2 vs 1 site	0.01 (-0.02 to 0.06)	0.44	-0.04 (-0.16 to 0.06)	0.40	-0.14 (-0.24 to -0.04)	0.005	-0.03 (-0.11 to 0.04)	0.36
Weight loss								
- ≥10% vs <10%	0.02 (-0.01 to 0.06)	0.12	-0.03 (-0.12 to 0.05)	0.46	0.04 (-0.04 to 0.12)	0.32	-0.11 (-0.18 to -0.05)	<0.001
Liver metastases								
- yes vs no	0.05 (0.02 to 0.09)	0.003	0.09 (-0.01 to 0.19)	0.06	-0.01 (-0.09 to 0.08)	0.90	-0.06 (-0.12 to 0.01)	0.06
Bone metastases								
- yes vs no	0.01 (-0.03 to 0.04)	0.86	0.02 (-0.07 to 0.12)	0.62	0.06 (-0.02 to 0.15)	0.17	0.04 (-0.02 to 0.11)	0.18
Brain metastases								
- yes vs no	-0.08 (-0.13 to -0.03)	0.001	-0.01 (-0.13 to 0.12)	0.91	0.01 (-0.09 to 0.13)	0.77	-0.01 (-0.09 to 0.07)	0.82
PD-L1 TPS								

- $\geq 1\%$ vs $< 1\%$	0.02 (-0.01 to 0.06)	0.21	-0.05 (-0.15 to 0.04)	0.27	0.10 (0.01 to 0.20)	0.019	0.02 (-0.04 to 0.09)	0.43
First line chemotherapy - carboplatin vs cisplatin-based	0.01 (-0.19 to 0.22)	0.89	0.12 (-0.09 to 0.34)	0.25	0.09 (-0.10 to 0.28)	0.35	-0.11 (-0.26 to 0.03)	0.11
First line chemotherapy - antimetabolite ^a vs taxane ^b	0.01 (-0.03 to 0.06)	0.51	0.05 (-0.06 to 0.16)	0.35	-0.01 (-0.10 to 0.10)	0.95	0.03 (-0.04 to 0.10)	0.45
Best response to previous chemotherapy - SD/CR/PR vs PD	-0.01 (-0.04 to 0.01)	0.36	-0.01 (-0.10 to 0.06)	0.65	-0.06 (-0.14 to 0.01)	0.08	-0.01 (-0.07 to 0.04)	0.64
Time from completion of previous chemotherapy - ≥ 3 months vs < 3 months	0.03 (-0.06 to -0.01)	0.038	0.03 (-0.04 to -0.11)	0.41	-0.02 (-0.10 to 0.05)	0.54	0.01 (-0.04 to 0.06)	0.64
Corticosteroids ^c - yes vs no	0.05 (0.01 to 0.10)	0.03	0.13 (0.01 to 0.25)	0.036	-0.07 (-0.18 to 0.03)	0.17	0.07 (-0.01 to 0.15)	0.08
Proton pump inhibitors - yes vs no	-0.01 (-0.05 to 0.03)	0.70	-0.08 (-0.19 to 0.03)	0.15	0.08 (-0.01 to 0.18)	0.10	0.02 (-0.05 to 0.10)	0.52
Antibiotics - yes vs no	-0.04 (-0.09 to -0.05)	0.03	-0.15 (-0.26 to -0.05)	0.004	-0.06 (-0.16 to 0.03)	0.19	0.01 (-0.06 to 0.07)	0.86
Statins - yes vs no	0.02 (-0.01 to 0.06)	0.25	0.05 (-0.05 to 0.15)	0.31	-0.03 (-0.12 to 0.05)	0.048	-0.04 (-0.10 to 0.02)	0.25
Metformin - yes vs no	-0.04 (-0.09 to 0.01)	0.09	-0.09 (-0.22 to 0.02)	0.12	0.06 (-0.05 to 0.17)	0.29	-0.07 (-0.16 to 0.01)	0.07

Abbreviations: log, logarithmic values; ECOG PS, Eastern Cooperative Oncology Group Performance Status; CR/PR, complete response/partial response; SD, stable disease; PD, progressive disease; PD-L1 TPS, programmed cell death-ligand 1 tumor proportion score.

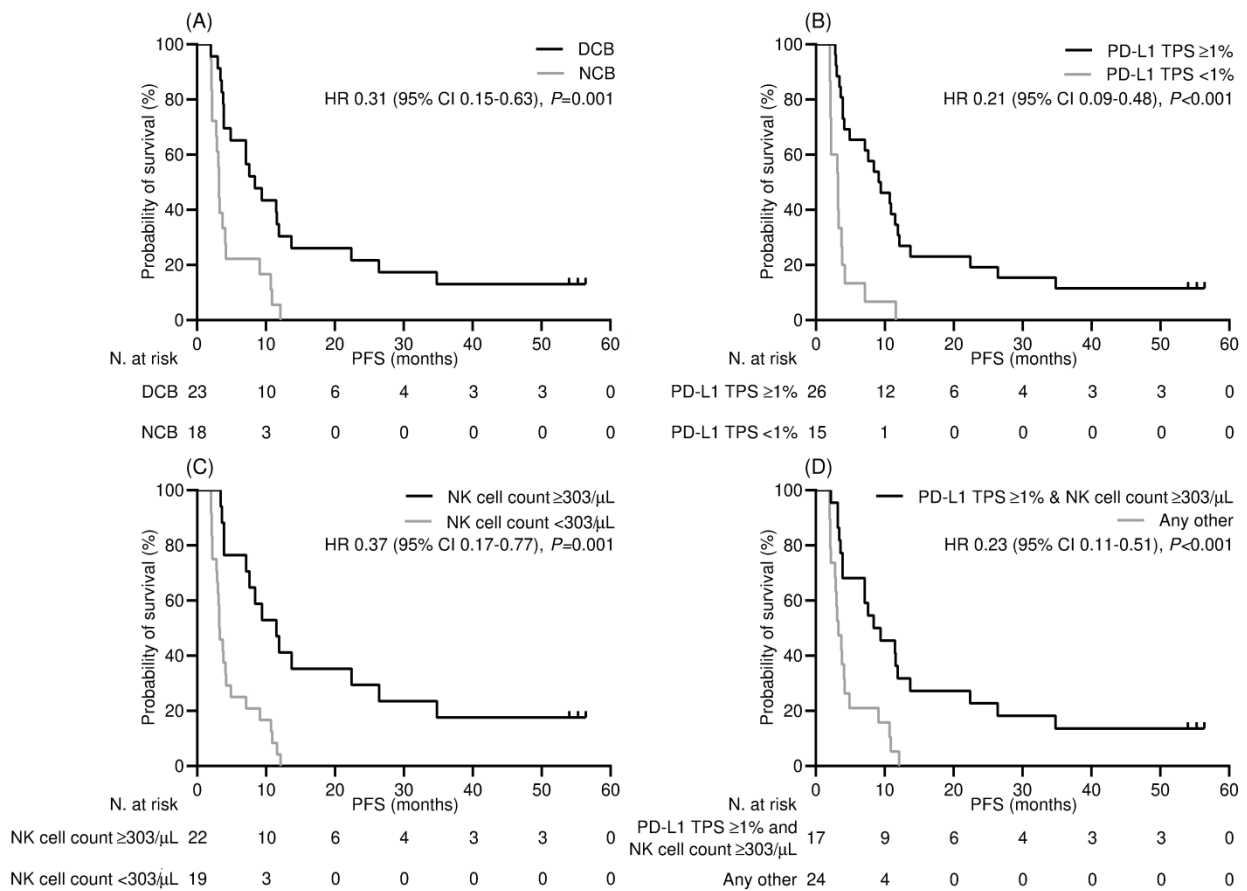
^a antimetabolite-based chemotherapy included regimens containing gemcitabine or pemetrexed; ^b taxane-based chemotherapy included regimens containing paclitaxel or docetaxel; ^c corticosteroids indicate < 10 mg prednisone equivalent daily. Time point 2 denotes post-treatment assessment.

Supplementary Figure 1. Gating strategy for flow cytometry assay



Gating 1: first gating on a plot of forward scatter ($CD45^+$) and side scatter (SSC-A) to detect the absolute value of lymphocytes; Gating 2: second gating on plot a forward scatter ($CD3^+$) and side scatter (SSC-A) to detect the absolute value of T lymphocytes and then gated on the $CD8^+$ and $CD4^+$ to detect the absolute values of T helper cells and T cytotoxic cells; Gating 3: third gating on a plot of forward scatter ($CD19^+$) and side scatter (SSC-A) to detect the absolute value of B lymphocytes and then gated on the $CD16^+CD56^+$ and $CD19^+$ to detect the absolute values of NK cells.

Supplementary Figure 2. Progression-free survival depending on significant clinical variables



A. DCB vs NCB; B. PD-L1 TPS: $\geq 1\%$ vs $< 1\%$; C. Post-treatment NK cell count: $\geq 303 \mu\text{L}$ vs $< 303 \mu\text{L}$; D. Co-presence of PD-L1 TPS $\geq 1\%$ and post-treatment NK cell count: $\geq 303 \mu\text{L}$ vs Any other
 Abbreviations: HR, hazard ratio; CI; confidence interval; DCB, durable clinical benefit; NCB, no clinical benefit; PD-L1, programmed cell death ligand-1 tumor proportion score