

ONLINE SUPPLEMENT

Subpopulations Of Circulating Cells With Morphological Features Of Malignancy Are Preoperatively Detected And Have Differential Prognostic Significance In Non-Small Cell Lung Cancer

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Supplementary Table S1. Single circulating tumor cell (sCTC) prevalence and non-small cell lung cancer (NSCLC) patients' characteristics

	N (%)	N sCTC≥1 (%)	N sCTC≥2 (%)	N sCTC≥3 (%)	N sCTC≥5 (%)
All cases	74 (100)	34 (45.9)	15 (20.3)	9 (12.2)	4 (5.4)
Sex					
Female	33 (44.6)	13 (39.4)	2 (6.1)	2 (6.1)	1 (3.0)
Male	41 (55.4)	21 (51.2)	13 (31.7)	7 (17.1)	3 (7.3)
<i>p-value</i>		.3542	.0081	.2831	.6237
Smoking habits					
Current smoker	25 (33.8)	12 (48.0)	7 (28.0)	4 (16.0)	1 (4.0)
Former smoker	32 (43.2)	16 (50.0)	8 (25.0)	5 (15.6)	3 (9.4)
Never-smoker	16 (21.6)	6 (37.5)	0	0	0
Missing	1 (1.4)	0	0	0	0
<i>p-value^a</i>		.5719	.0308	.1915	.5699
Tumor stage					
IA	27 (36.5)	12 (44.4)	4 (14.8)	3 (11.1)	1 (3.7)
IB	5 (6.8)	1 (20.0)	0	0	0
IIA	4 (5.4)	4 (100)	2 (50.0)	2 (50.0)	0
IIB	16 (21.6)	7 (43.8)	3 (18.8)	1 (6.3)	0
IIIA	11 (14.9)	6 (54.5)	4 (36.4)	1 (9.1)	1 (9.1)
IIIB	6 (8.1)	2 (33.3)	1 (16.7)	1 (16.7)	1 (16.7)
IIIC	0	0	0	0	0
IVA	5 (6.8)	2 (40.0)	1 (20.0)	1 (20.0)	1 (20.0)
<i>p-value^b</i>		>.9999	.3551	>.9999	.0760
Histological subtype					
Adenocarcinoma	55 (74.3)	25 (45.5)	10 (18.2)	5 (9.1)	3 (5.5)
Squamous cell carcinoma	16 (21.6)	8 (50.0)	5 (31.3)	4 (25.0)	1 (6.3)
Other	2 (2.7)	1 (50.0)	0	0	0
Missing	1 (1.4)	0	0	0	0
<i>p-value^c</i>		.7825	.3030	.1081	>.9999
Grading					
G1	4 (5.4)	2 (50.0)	0	0	0
G2	44 (59.5)	17 (38.6)	7 (15.9)	4 (9.1)	2 (4.5)
G3	24 (32.4)	14 (58.3)	8 (33.3)	5 (20.8)	2 (8.3)
G4	0	0	0	0	0
Missing	2 (2.7)	1 (50.0)	0	0	0
<i>p-value^d</i>		.1435	.1212	.1492	.5965

^a current/former versus never-smokers

^b stage I/II versus III/IV

^c adenocarcinoma versus squamous cell carcinoma

^d G1/G2 versus G3/G4

Supplementary Table S1. Single circulating tumor cell (sCTC) prevalence and non-small cell lung cancer (NSCLC) patients' characteristics (*continued*)

	N (%)	N sCTC≥1 (%)	N sCTC≥2 (%)	N sCTC≥3 (%)	N sCTC≥5 (%)
Visceral pleura invasion					
PL0	56 (75.7)	26 (46.4)	13 (23.2)	8 (14.3)	3 (5.4)
PL1	8 (10.8)	5 (62.5)	1 (12.5)	1 (12.5)	1 (12.5)
PL2	5 (6.8)	1 (20.0)	0	0	0
PL3	2 (2.7)	1 (50.0)	1 (50.0)	0	0
Missing	3 (4.1)	1 (33.3)	0	0	0
<i>p-value</i> ^e		>.9999	>.5001	.6730	>.9999
Peritumoral neoplastic angioinvasion					
Absent	66 (89.2)	32 (48.5)	14 (21.2)	9 (13.6)	4 (6.1)
Present	6 (8.1)	1 (16.7)	1 (16.7)	0	0
Missing	2 (2.7)	1 (50.0)	0	0	0
<i>p-value</i>		.2088	>.9999	>.9999	>.9999
Lymph-node status					
Negative	48 (64.9)	22 (45.8)	10 (20.8)	6 (12.5)	2 (4.2)
Positive	25 (33.8)	11 (44.0)	5 (20.0)	3 (12.0)	2 (8.0)
Missing	1 (1.4)	0	0	0	0
<i>p-value</i>		>.9999	>.9999	>.9999	.6027

^e PL0 vs PL1/PL2/PL3

Abbreviations: hetCLU, heterotypic cluster; homCLU, homotypic CTC cluster

Supplementary Table S2. Circulating tumor cell cluster (cCTC) status and NSCLC patients' characteristics

	N (%)	N cCTC+ve (%)	N hetCLU+ve (%)	N homCLU+ve (%)
All cases	74 (100)	24 (32.4)	23 (31.1)	2 (2.7)
Sex				
Female	33 (44.6)	10 (30.3)	10 (30.3)	0
Male	41 (55.4)	14 (34.1)	13 (31.7)	2 (4.9)
<i>p-value</i>		.8055	>.9999	.4991
Smoking habits				
Current smoker	25 (33.8)	12 (48.0)	11 (44.0)	1 (4.0)
Former smoker	32 (43.2)	8 (25.0)	8 (25.0)	1 (3.1)
Never-smoker	16 (21.6)	4 (25.0)	4 (25.0)	0
Missing	1 (1.4)	0	0	0
<i>p-value</i>		.5553	.7616	>.9999
Tumor stage				
IA	27 (36.5)	9 (33.3)	9 (33.3)	0
IB	5 (6.8)	2 (40.0)	2 (40.0)	0
IIA	4 (5.4)	1 (25.0)	1 (25.0)	0
IIB	16 (21.6)	6 (37.5)	5 (31.3)	2 (12.5)
IIIA	11 (14.9)	2 (18.2)	2 (18.2)	0
IIIB	6 (8.1)	1 (16.7)	1 (16.7)	0
IIIC	0	0	0	0
IVA	5 (6.8)	3 (60.0)	3 (60.0)	0
<i>p-value</i>		.5974	.7858	>.9999
Histology				
Adenocarcinoma	55 (74.3)	18 (32.7)	18 (32.7)	0
Squamous cell carcinoma	16(21.6)	5 (31.3)	4 (25.0)	2 (12.5)
Other	2 (2.7)	1 (50.0)	1 (50.0)	0
Missing	1 (1.4)	0	0	0
<i>p-value</i>		>.9999	.7603	.0483
Grading				
G1	4 (5.4)	2 (50.0)	2 (50.0)	0
G2	44 (59.5)	14 (31.8)	14 (31.8)	1 (2.3)
G3	24 (32.4)	8 (33.3)	7 (29.2)	1 (4.2)
G4	0	0	0	0
Missing	2 (2.7)	0	0	0
<i>p-value</i>		>.9999	.7935	>.9999

^a current/former versus never-smokers

^b stage I/II versus III/IV

^c adenocarcinoma versus squamous cell carcinoma

^d G1/G2 versus G3/G4

Abbreviations: hetCLU, heterotypic cluster; homCLU, homotypic CTC cluster

Supplementary Table S2. Circulating tumor cell cluster (cCTC) status and NSCLC patients' characteristics (*continued*)

	N (%)	N cCTC+ve (%)	N hetCLU+ve (%)	N homCLU+ve (%)
Visceral pleura invasion				
PL0	56 (75.7)	16 (28.6)	15 (26.8)	2 (3.6)
PL1	8 (10.8)	2 (25.0)	2 (25.0)	0
PL2	5 (6.8)	3 (60.0)	3 (60.0)	0
PL3	2 (2.7)	2 (100)	2 (100)	0
Missing	3 (4.1)	1 (33.3)	1 (33.3)	0
<i>p-value</i>		.2007	.2075	>.9999
Peritumoral neoplastic angi invasion				
Absent	66 (89.2)	24 (36.4)	23 (34.8)	2 (3.0)
Present	6 (8.1)	0	0	0
Missing	2 (2.7)	0	0	0
<i>p-value</i>		.1692	.1674	>.9999
Lymph-node status				
Negative	48 (64.9)	17 (35.4)	16 (33.3)	1 (2.1)
Positive	25 (33.8)	7 (28.0)	7 (28.0)	1 (4.0)
Missing	1 (1.4)	0	0	0
<i>p-value</i>		.6055	.7920	>.9999

° PL0 vs PL1/PL2/PL3

Abbreviations: hetCLU, heterotypic cluster; homCLU, homotypic CTC cluster

Supplementary Table S3. Recurrence-free survival probability in patients with operable stage I-II NSCLC according to standard clinico-pathological parameters and CTC status

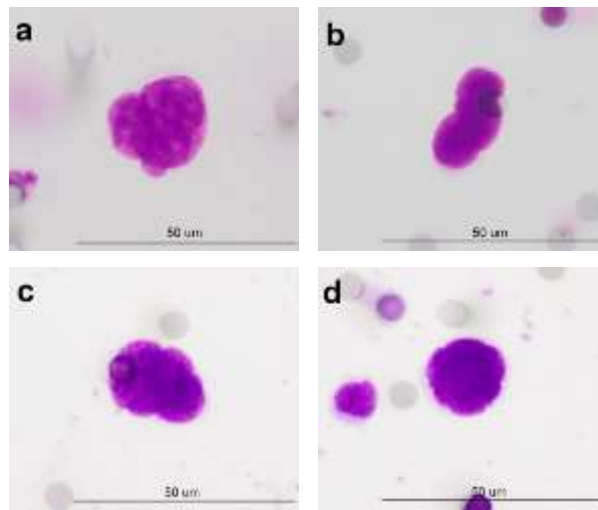
Variable	N cases	N recurrence events	Survival proportion (%)	HR 95% (CI)	p-value
pStage					
I	30	7	70	2.19 (0.65-7.36)	.1452
II	14	6	51		
Grading					
G1/G2	33	9	70	1.52 (0.41-5.57)	.4820
G3/G4	11	4	51		
Lymph-node status					
Negative	38	11	65	1.47 (0.26-8.36)	.1092
Positive	6	2	56		
CTC status					
Negative	18	3	82	2.27 (0.75-6.86)	.2003
Positive	26	10	55		
sCTC count					
<2	36	7	77	5.15 (1.10-24.33)	.0009
≥2	8	6	15		
sCTC count					
<3	39	10	69	3.99 (0.47-33.57)	.0216
≥3	5	3	27		
hetCLU status					
Negative	29	7	73	1.42 (0.46-4.35)	.5267
Positive	15	6	55		

Abbreviations. “HR”, hazard ratio; “CI”, confidence interval; “CTC”, circulating tumor cell(s); “hetCLU”, heterotypic circulating tumor cell clusters.

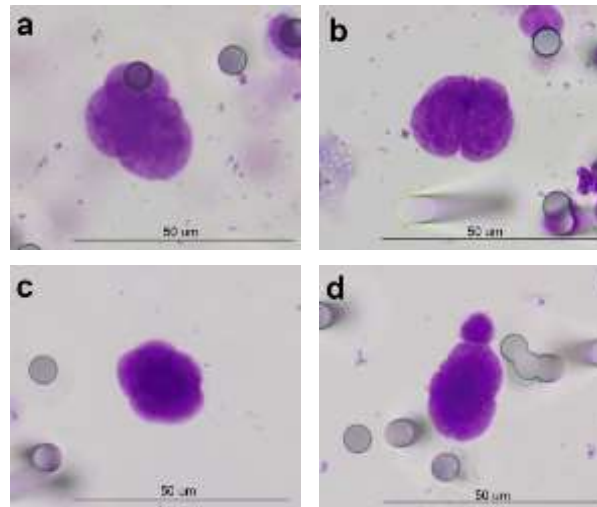
Supplementary Table S4. Recurrence-free survival probability in patients with operable stage III-IV NSCLC according to standard clinico-pathological parameters and CTC status

Variable	N cases	N recurrence events	Survival proportion (%)	HR 95% (CI)	p-value
pStage					
III	13	8	33		
IV	5	5	0	2.78 (0.68-11.33)	.0537
Grading					
G1/G2	8	5	38		
G3/G4	8	6	18	1.33 (0.41-4.35)	.6261
CTC status					
Negative	6	3	44		
Positive	12	10	11	3.12 (1.05-9.27)	.0573
sCTC count					
<2	12	9	21		
≥2	6	4	25	1.10 (0.32-3.49)	.9223
sCTC count					
<3	15	11	19		
≥3	3	2	33	0.73 (0.19-2.82)	.6693
hetCLU status					
Negative	13	8	31		
Positive	5	5	0	3.44 (0.76-15.50)	.0129

Abbreviations. “HR”, hazard ratio; “CI”, confidence interval; “CTC”, circulating tumor cell(s); “hetCLU”, heterotypic circulating tumor cell clusters.



Supplementary Figure S1. Naked nuclei detected in the blood of lung cancer-free individuals. Images depict naked nuclei, i.e. without apparent cytoplasm, with the longer diameter $>20\ \mu\text{m}$ and irregular border, sometimes (**a**, **c**) overlapped, (**b**) oblong or (**b**, **d**) hyperchromatic, on porous membranes stained with May-Grünwald Giemsa. Objective magnification 40x.



Supplementary Figure S2. Naked nuclei detected in the blood of non-small cell lung cancer patients. Images depict naked nuclei, i.e. without apparent cytoplasm, with the longer diameter $>20\ \mu\text{m}$ and irregular border, sometimes (a) overlapped, (b) juxtaposed, (b-d) hyperchromatic, and (d) clustered with other smaller nuclei consistent with possible leukocytes, on porous membranes stained with May-Grünwald Giemsa. Objective magnification 40x.