Supplementary

Table S1 Characteristics of patients in discovery cohort

Characteristics	All patients (n=44)	CYFRA >3.0 ng/mL (n=13, 29.5%)	CYFRA ≤3.0 ng/mL (n=31, 70.5%)	P value
Age (years), median (range)	70 (43–79)	65 (54–77)	70 (43–79)	0.65
Sex, n (%)				0.07
Male	31 (70.5)	12 (92.3)	19 (61.3)	
Female	13 (29.5)	1 (7.7)	12 (38.7)	
ECOG PS, n (%)				0.75
0	25 (56.8)	8 (61.5)	17 (54.8)	
1, 2, 3	19 (43.2)	5 (38.5)	14 (45.2)	
Stage, n (%)				0.65
III/IV	38 (86.4)	12 (92.3)	26 (83.9)	
Recurrence	6 (13.6)	1 (7.7)	5 (16.1)	
Oncogenic driver, n (%)				1
EGFR mutation positivity	1 (2.3)	0 (0.0)	1 (3.2)	
ALK rearrangement	0 (0.0)	0 (0.0)	0 (0.0)	
Smoking status, n (%)				0.40
Current/former	36 (81.8)	12 (92.3)	24 (77.4)	
Never	8 (18.2)	1 (7.7)	7 (22.6)	
Histology, n (%)				
Adenocarcinoma	25 (56.8)	4 (30.8)	21 (67.7)	0.04
Squamous cell carcinoma	12 (27.3)	5 (38.5)	7 (22.6)	0.30
Others	7 (15.9)	4 (30.8)	3 (9.7)	
PD-L1 TPS, n (%)				0.13
≥50%	11 (25.0)	1 (7.7)	10 (32.3)	
1–49%	17 (38.6)	9 (69.2)	8 (25.8)	
<1%	16 (36.4)	3 (23.1)	13 (41.9)	
Regimen, n (%)				1
Pembrolizumab regimen	37 (84.1)	11 (84.6)	26 (83.9)	
Atezolizumab regimen	7 (15.9)	2 (15.4)	5 (16.1)	
Response assessment				–
CR	0 (0.0)	0 (0.0)	0 (0.0)	
PR	19 (43.2)	5 (38.5)	14 (45.2)	
SD	15 (34.1)	5 (38.5)	10 (32.3)	
PD	4 (9.1)	2 (15.4)	2 (6.5)	
NE	6 (13.6)	1 (7.7)	5 (16.1)	
Overall response rate (%) (95% CI)	43.2 (28.3–59.0)	38.5 (13.9–68.4)	45.2 (27.3–64)	0.75
Disease control rate (%) (95% CI)	77.3 (62.2–88.5)	76.9 (46.2–95)	77.4 (58.9–90.4)	1

CYFRA, cytokeratin fragment; ECOG PS, Eastern Cooperative Oncology Group performance status; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; PD-L1 TPS, programmed death ligand 1 tumor progression score; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluated; CI, confidence interval.

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Table S2 Characteristics of patients in validation cohort

Characteristics	All patients (n=77)	CYFRA >3.0 ng/mL (n=43, 55.8%)	CYFRA ≤3.0 ng/mL (n=34, 44.2%)	P value
Age (years), median (range)	70 (44–86)	70 (47–85)	73 (44–86)	0.13
Sex, n (%)				0.74
Male	67 (87.0)	38 (88.4)	29 (85.3)	
Female	10 (13.0)	5 (11.6)	5 (14.7)	
ECOG PS, n (%)				0.24
0	30 (39.0)	14 (32.6)	16 (47.1)	
1, 2, 3	47 (61.0)	29 (67.4)	18 (52.9)	
Stage, n (%)				0.19
III/IV	76 (98.7)	43 (100.0)	32 (94.1)	
Recurrence	1 (1.3)	0 (0.0)	2 (5.9)	
Oncogenic driver, n (%)				0.32
EGFR mutation positivity	4 (5.2)	1 (2.3)	3 (8.8)	
ALK rearrangement	0 (0.0)	0 (0.0)	0 (0.0)	
Smoking status, n (%)				0.03
Current/former	65 (84.4)	40 (93.0)	25 (73.5)	
Never	12 (15.6)	3 (7.0)	9 (26.5)	
Histology, n (%)				
Adenocarcinoma	49 (63.6)	27 (62.8)	22 (64.7)	1
Squamous cell carcinoma	19 (24.7)	12 (27.9)	7 (20.6)	0.60
Others	9 (11.7)	4 (9.3)	5 (14.7)	
PD-L1 TPS, n (%)				0.63
≥50%	26 (33.8)	16 (37.2)	10 (29.4)	
1–49%	29 (37.7)	19 (44.2)	10 (29.4)	
<1%	22 (28.6)	8 (18.6)	14 (41.2)	
Regimen, n (%)				0.20
Pembrolizumab regimen	56 (72.7)	34 (79.1)	22 (64.7)	
Atezolizumab regimen	21 (27.3)	9 (20.9)	12 (35.3)	
Response assessment, n (%)				
CR	2 (2.6)	0 (0.0)	2 (5.9)	
PR	49 (63.6)	27 (62.8)	22 (64.7)	
SD	15 (19.5)	8 (18.6)	7 (20.6)	
PD	6 (7.8)	4 (9.3)	2 (5.9)	
NE	5 (6.5)	4 (9.3)	1 (2.9)	
Overall response rate (%) (95% CI)	66.2 (54.6–76.6)	62.8 (46.7–77.0)	70.6 (52.5–84.9)	0.63
Disease control rate (%) (95% CI)	85.7 (75.9–92.6)	81.4 (66.6–91.6)	91.2 (76.3–98.1)	0.33

CYFRA, cytokeratin fragment; ECOG PS, Eastern Cooperative Oncology Group performance status; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; PD-L1 TPS, programmed death ligand 1 tumor progression score; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluated; CI, confidence interval.

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Table S3 Cox proportional-hazards models for time to progression-free survival in patients with non-small cell lung cancer in discovery cohort

Items (comparator)	Univariate		Multivariate	
	HR (95% CI)	P value	HR (95% CI)	P value
CYFRA 21-1 >3.0 ng/mL (*vs.* ≤3.0 ng/mL)	2.51 (1.19–5.32)	0.02	2.97 (1.24–7.13)	0.02
CEA >9.7 ng/mL (*vs.* ≤9.7 ng/mL)	1.37 (0.65–2.87)	0.40	–	–
Age ≥75 years (*vs.* <75 years)	1.66 (0.67–4.07)	0.27	2.05 (0.79–5.27)	0.14
Male sex (*vs.* female sex)	1.04 (0.49–2.22)	0.91	0.90 (0.34–2.36)	0.83
Smoker (*vs.* never smoker)	0.87 (0.36–2.14)	0.77	0.71 (0.23–2.20)	0.55
PD-L1 TPS ≥50% (*vs.* <50%)	0.60 (1.42–11.3)	0.25	0.85 (0.34–2.11)	0.72
Squamous (*vs.* non-squamous)	1.12 (0.52–2.44)	0.77	1.02 (0.44–2.38)	0.96
Pembrolizumab regimen (vs. atezolizumab regimen)	1.33 (0.46–3.83)	0.59	–	–

HR, hazard ratio; CI, confidence interval; CYFRA, cytokeratin fragment; CEA, carcinoembryonic antigen; PD-L1 TPS, programmed death ligand 1 tumor progression score.

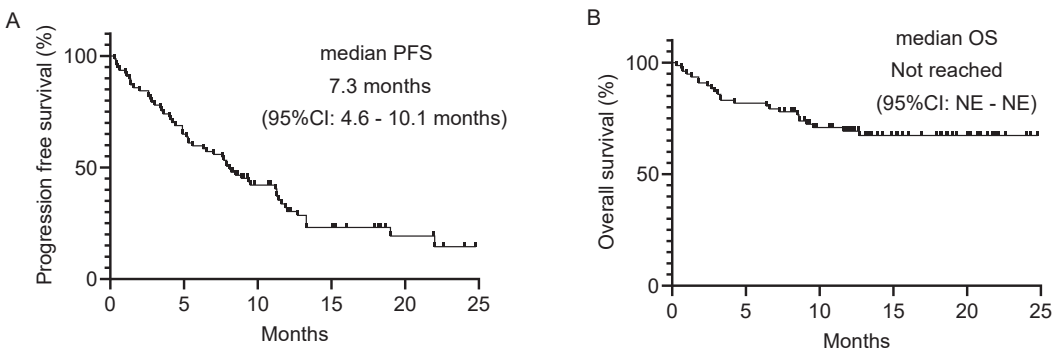

Figure S1 PFS (A) and OS (B) of patients treated with combined chemoimmunotherapy in discovery cohort. PFS, progression-free survival; CI, confidence interval; OS, overall survival; NE, not evaluated.

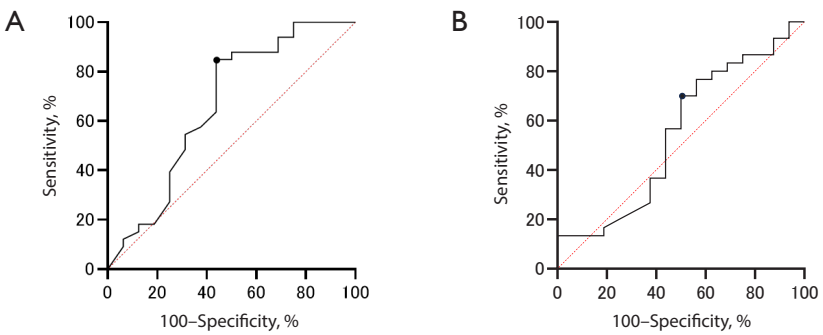

Figure S2 Receiver operator characteristic curves to determine the optimal cutoff values for CYFRA 21-1 (A) and CEA (B). CYFRA, cytokeratin fragment; CEA, carcinoembryonic antigen.

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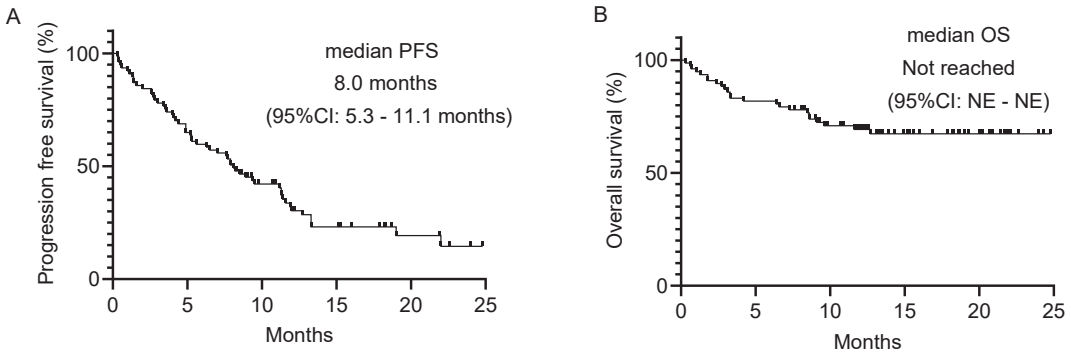

Figure S3 PFS (A) and OS (B) of patients treated with combined chemoimmunotherapy in validation cohort. PFS, progression free survival; CI, confidence interval; OS, overall survival; NE, not evaluated.

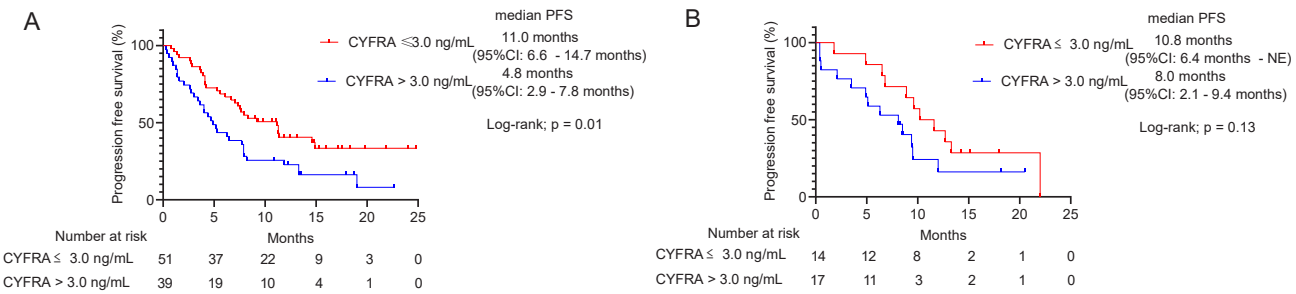

Figure S4 PFS based on the status of CYFRA 21-1 in patients according to the histology. PFS was stratified according to non-squamous (A), and squamous (B). PFS, progression-free survival; CI, confidence interval; CYFRA, cytokeratin fragment; NE, not evaluated.

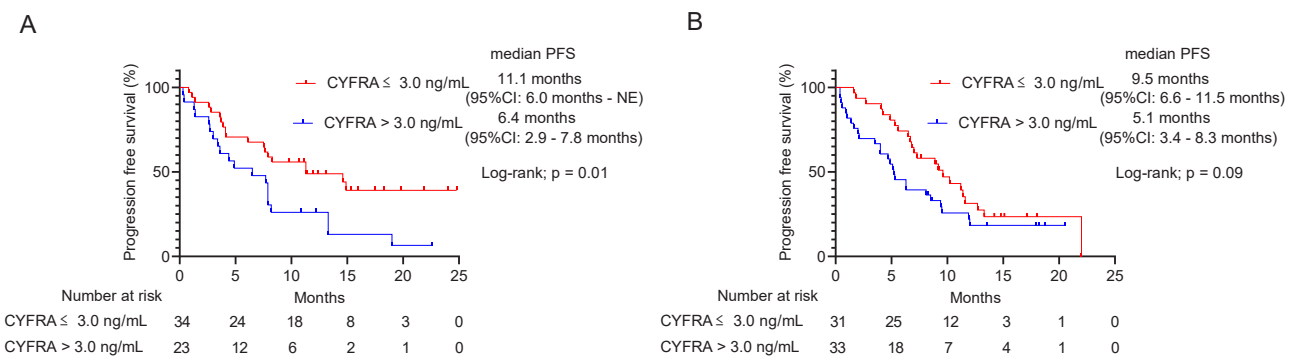

Figure S5 PFS based on the status of CYFRA 21-1 in patients according to the received chemotherapy regimens. PFS was stratified according to receiving the pemetrexed regimen (A) and paclitaxel or nab-paclitaxel regimen (B). PFS, progression-free survival; CI, confidence interval; NE, not evaluated; CYFRA, cytokeratin fragment.

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