Ther Adv Med Oncol
2020, Vol. 12: 1–13
DOI: 10.1177/1758835920952994

CEA and CYFRA 21-1 as prognostic biomarker and as a tool for treatment monitoring in advanced NSCLC treated with immune checkpoint inhibitors

Filippo G. Dall'Olio, Francesca Abbati, Francesco Facchinetti, Maria Massucci, Barbara Melotti, Anna Squadrilli, Sebastiano Buti, Francesca Formica, Marcello Tiseo* and Andrea Ardizzoni*

Abstract

Aims: To assess prognostic value of pre-therapy carcinoembryonic antigen (CEA) and cytokeratin-19 fragments (CYFRA 21-1) blood levels in non-small cell lung cancer (NSCLC) patients treated with immune-checkpoint inhibitors (ICIs) and their early change as predictor of benefit.

Materials and methods: This is a retrospective cohort study including patients with stage IIIB–IV NSCLC who received anti PD-1/PD-L1 in first or advanced lines of therapy in two institutions. A control cohort of patients treated only with chemotherapy has been enrolled as well.

Results: A total of 133 patients treated with nivolumab or atezolizumab were included in the test set, 74 treated with pembrolizumab first line in the validation set and 89 in the chemotherapy only cohort. CYFRA 21-1 >8 ng/mL was correlated with overall survival (OS) in the test set, validation set and in univariate and multivariate analysis (pooled cohort hazard ratio (HR) 1.90, 95% confidence interval (CI) 1.24–2.93, p 0.003). Early 20% reduction after the third cycle was correlated with OS for CEA (HR 0.12; 95% CI 0.04–0.33; $p < 0.001$), and for CYFRA 21-1 (HR 0.19; 95% CI 0.07–0.55; p 0.002)

Conclusions: CYFRA 21-1 pre-therapy assessment provides clinicians with relevant prognostic information about patients treated with ICI. CEA and CYFRA 21-1 repeated measures could be useful as an early marker of benefit.

Keywords: advanced lung cancer, carcinoembryonic antigen, CEA, CYFRA, cytokeratin 19 fragment, immune checkpoint, NSCLC, predictive, prognostic, serum tumor markers

Received: 9 April 2020; revised manuscript accepted: 3 August 2020.

Correspondence to:
Filippo G. Dall'Olio
Policlinico Sant'Orsola Malpighi, Division of Oncology, University of Bologna, Via Giuseppe Massarenti, 9, Bologna, 40138, Italy
filippogdallolio@gmail.com

Francesca Abbati
Maria Massucci
Barbara Melotti
Francesca Formica
Andrea Ardizzoni
Oncologia Medica, Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italia

Francesco Facchinetti
INSERM U981, Gustave Roussy Cancer Campus, Université Paris Saclay, Villejuif, France

Anna Squadrilli
Sebastiano Buti
Medical Oncology Unit, University Hospital of Parma, Parma, Italy

Marcello Tiseo
Medical Oncology Unit, University Hospital of Parma, Parma, Italy

Department of Medicine and Surgery, University of Parma, Parma, Italy

*These authors equally contributed to the work.

Introduction

Immune checkpoint inhibitors (ICIs) have changed clinical practice in non-small cell lung cancer (NSCLC) over the last few years.

Currently, nivolumab, pembrolizumab (anti-PD-1) and atezolizumab (anti-PD-L1) have been approved by the Food and Drug Administration and the European Medicines Agency for second line treatment of advanced NSCLC patients.[1–4] Pembrolizumab has been approved for use in first-line, both individually,[5] for patients with high PD-L1 expression, and in combination with platinum-based chemotherapy, regardless of PD-L1 expression,[6] while atezolizumab has been approved for use in combination with carboplatin, paclitaxel and bevacizumab.[7]

PD-L1 expression assessed by immunohistochemistry represents the only validated predictive marker for immunotherapy, as shown by the impressive benefit derived from pembrolizumab

in the first-line setting of strong PD-L1 (⩾ 50% of tumor cells) tumors[5] and by the incremental benefit in pre-treated patients according the expression level.[2–4] Nevertheless, responses in 'negative' and progressions in 'strongly positive' cases are also observed. Moreover, PD-L1 expression analysis on small biopsies has been shown to be impaired by the high heterogeneity both across different anatomical regions and within single cancer-tissue samples, raising doubts about its reliability as a predictive factor.[8,9]

Tumor mutational burden has been correlated with disease outcomes,[10,11] but its analysis is expensive and time consuming and, therefore, difficult to incorporate into clinical practice.[12] Moreover recent evidence questions its reliability, at least in first line combination with chemotherapy.[13,14]

An important unmet need in this field is the identification of predictive factors that could help in the selection of those patients who are more likely to benefit from ICIs.

Serum tumor markers (STMs) such as carcinoembryonic antigen (CEA) and cytokeratin-19 fragments (CYFRA 21-1) have been investigated as prognostic and predictive factors and for treatment monitoring in NSCLC patients treated with chemotherapy.[15–17] There is nevertheless scarce and conflicting evidence on their possible prognostic role within the contest of immunotherapy,[18–20] although some reports do support their use in treatment monitoring.[20–22]

Materials and methods

Patients

This is a retrospective study on a cohort of 283 patients with pathologically proven stage IIIB–IV NSCLC for whom baseline serum tumor markers blood levels were available.

Initially, patients treated with nivolumab or atezolizumab as second or further line of therapy between August 2015 and May 2019 were included in a test set to establish the potential of CEA and CYFRA 21-1 as prognostic factors of outcome in patients treated with immunotherapy and to identify the best cutoff levels. The hypothesis was then validated in a second set that included patients treated with first line pembrolizumab from July 2017 to January 2020.

To determine whether CEA and CYFRA 21-1 levels were generally prognostic or, rather, specifically predictive for immunotherapy benefit, a control cohort of patients with advanced NSCLC treated exclusively with chemotherapy from January 2011 to December 2012 at Bologna University Hospital was also evaluated.

This study was approved by the local ethical committee ('Comitato Etico Area Vasta Emilia Centro', Approval number 404/2019). All patients alive at the moment of ethics committee approval had to provide written, informed consent. The committee waived the requirement to obtain informed consent for those who were already dead. The end of the observation period for this study was January 2020.

REMARK guidelines were followed for study design, conduct, analysis and evaluation of results[23]

Data on clinical and demographics characteristics including age, sex, number of prior systemic chemotherapies, histological type, PD-L1 expression, performance status based on ECOG scale, smoking history, presence or absence of liver, bone and brain metastasis, and neutrophil, lymphocyte count and each of the serum markers CEA and CYFRA at the beginning of immunotherapy (from day –28 to day 1 of the first cycle) and after three cycles (±1) were extracted from medical records.

Median overall survival (OS) was chosen as primary endpoint; disease control rate (DCR) was also analysed. OS was measured from the first ICI administration to death from any cause. Tumor response was assessed by computed tomography (CT) scan according to RECIST version 1.1 criteria. Radiologic assessments were performed with CT scans every 8–12 weeks.

Methods

CEA was measured by using chemiluminescence test, ACCESS CEA, instrument DXI (Beckman Coulter, Brea, Los Angeles, USA). CYFRA 21-1 was measured by using Kryptor compact plus (Thermo Fisher Scientific B.R.A.H.M.S, Asnieres, France) based on time resolved amplified cryptate emission. The assay of each marker was performed following the directions given by the manufacturer. Results were expressed in nanograms per milliliter (ng/mL). The upper

limit of normality (ULN) is 5.0 ng/mL for CEA and 3.3 ng/mL for CYFRA 21-1. These were calculated as the mean plus two standard deviations of the two tumor markers in healthy controls according to published reports. Based on available literature, a threshold of 20% reduction was selected as a STM response both for CEA and CYFRA 21-1.[15]

Statistical analyses

Clinical and pathological information was summarized using summary statistics.

Patient characteristics were compared using χ^2 or Fisher exact test for discrete variables and the unpaired *t* test, Wilcoxon sign-rank test when appropriate. OS was estimated using the Kaplan–Meier method. Median follow–up was calculated with reverse Kaplan–Meier method. Receiver operating characteristic (ROC) curve was used to find the best cut-off in the test set, using the status at 12 months (dead or alive) as state variable. Cox proportional hazard model was used to evaluate factors independently associated with OS, while logistic regression was used for DCR. Variables included in the final multivariate model were selected according to their clinical relevance and statistical significance in a univariate analysis ($p \leqslant 0.10$). The multivariate model was designed using the backward stepwise method. Internal validation of the final multivariate model for OS and for DCR was performed on the ICI pooled cohort with a bootstrap sample procedure ($n = 1000$ samples). Performance of the final model was further quantified by the Harrell C index and validated with bootstrap resampling procedure to calculate bias corrected C-index.

The *p* value was considered significant when inferior to 0.05. Statistical analysis was performed using RStudio Version 1.2.1335.

Results

Baseline characteristics are summarized in Table 1.

The main characteristics of the three populations (test, validation and chemotherapy set) are comparable (excluding line of treatment and PD-L1 status). Relationship of CEA and CYFRA 21-1 with other clinic pathological data is listed in Supplemental Material Table A.1 and A.2 online.

Test set

We retrospectively identified 147 consecutive patients that had been treated with nivolumab or atezolizumab in our institutions. Of those, for 14 patients baseline STMs were not available and they were therefore excluded, while the remaining 133 were analysed.

Median follow-up duration was 34.8 months. Median OS was 6.4 months (95% confidence interval (CI) 3.0–7.8 months). The ROC curve showed an area under the curve of 0.816 for CYFRA 21-1 and 0.664 for CEA. Based on the ROC curve, we chose 8.0 ng/mL as cut-off for both CYFRA 21-1 (sensibility 65%, specificity 82%) and CEA (sensibility 64%, specificity 71%) (Supplemental Figure A.1). Moreover, based on previous experience, we chose 4.0 as cut-off for the neutrophil/lymphocyte ratio.[24]

The median OS for patients with CYFRA 21-1 >8 ng/mL was 2.7 months (95% CI 1.2–4.2) *versus* 16.6 months (95% CI 10.1–23.1; Supplemental Figure A.2) in patients with CYFRA 21-1 values ⩽8 ng/mL (hazard ratio (HR) 3.01; 95% CI 1.93–4.69; $p < 0.001$).

CYFRA 21-1 above 8.0 ng/mL was correlated with a worse prognosis in multivariate analysis (HR 2.30, 95% CI 1.41–3.73, *p* 0.001), while CEA levels >8 ng/mL were not correlated with prognosis (*p* 0.238). (Supplemental Table A.3).

DCR resulted significantly lower in patients with CYFRA 21-1 >ULN (30% *versus* 55%, OR 0.34; 95% CI 0.14–0.82; *p* 0.017; data not shown).

Validation set

A total of 74 consecutive patients treated with first line pembrolizumab with CYFRA 21-1 baseline serum levels were analysed. Median follow-up was 13.9 months. Their main characteristics are listed in Table 1 and are comparable to those in the test set.

Median OS was 5.1 (95% CI 0.10–11.6) for CYFRA 21-1 >8 ng/mL *versus* 21.5 months (95% CI 10.4–32.6) for CYFRA 21-1 ⩽8.0 ng/mL. CYFRA 21-1 >8 was correlated with OS in multivariate analysis (HR 2.25; 95% CI 1.00–5.06; p 0.049) (Supplemental Table A.4 and Figure A.3).

DCR resulted lower in patients with CYFRA 21-1 >8 ng/mL, albeit formally non-significant at

Table 1. Clinicopathological characteristics of patients included in training set, validation set and chemotherapy control group.

	Training set	Validation set	Chemotherapy
	n = 133	*n* = 74	*n* = 98
Sex			
Male	94 (71%)	48 (65%)	58 (58%)
Female	39 (29%)	26 (35%)	42 (42%)
Median age			
	69 years	70.5 years	65 years
Smoker			
Former	71 (53%)	33 (44%)	50 (51%)
Current	46 (35%)	37 (50%)	38 (39%)
Never	16 (12%)	4 (6%)	10 (10%)
Performance status (ECOG)			
0–1	102 (77%)	60 (81%)	93 (95%)
2	31 (23%)	14 (19%)	5 (5%)
Drug			
Nivolumab	111 (83%)	0 (0%)	–
Pembrolizumab	0 (0%)	74 (100%)	–
Atezolizumab	22 (17%)	0 (0%)	–
Histology			
Squamous	39 (29%)	12 (16%)	20 (20%)
Non-squamous	94 (71%)	62 (84%)	78 (80%)
Stage			
IIIB	17 (13%)		15 (16%)
IV	116 (87%)	74 (100%)	83 (84%)
Line of therapy			
1st	0 (0%)	74 (100%)	98 (100%)
2nd	88 (66%)	0 (0%)	
>2nd	45 (34%)	0 (0%)	
Liver metastasis			
Yes	29 (22%)	6 (8%)	13 (13%)
No	104 (77%)	68 (92%)	85 (87%)

(Continued)

Table 1. (Continued)

	Training set	Validation set	Chemotherapy
	n **= 133**	***n*** **= 74**	***n*** **= 98**
Bone metastasis			
Yes	39 (29%)	21 (28%)	36 (36%)
No	94 (71%)	53 (72%)	62 (64%)
Brain metastasis			
Yes	24 (18%)	13 (18%)	13 (13%)
No	109 (82%)	61 (82%)	85 (87%)
Neutrophil/lymphocyte ratio ⩾4			
Yes	54 (41%)	34 (46%)	46 (47%)
No	79 (59%)	40 (54%)	52 (53%)
CEA >8			
Yes	73 (55%)	33 (45%)	46 (47%)
No	59 (44%)	33 (45%)	48 (49%)
N/A	1 (1%)	8 (10%)	4 (4%)
CYFRA >8			
Yes	54 (41%)	31 (42%)	27 (27%)
No	66 (50%)	43 (58%)	70 (71%)
N/A	13 (9%)		1 (1%)
PD-L1			
<1%	22 (17%)		
⩾1%	29 (22%)	74 (100%)	
NA	82 (61%)		98 (100%)

CEA, carcinoembryonic antigen; CYFRA 21-1, cytokeratin-19 fragments

0.05 level (53% *versus* 80%; OR 0.29; 95% CI 0.08–1.03; p 0.056; data not shown).

ICI pooled cohort

Median follow-up was 17.4 months (95% CI 11.5–23.3). Median CYFRA 21-1 level was 6.2 (range 0.0–1432.0) and was >8 ng/mL in 85 patients (41%).

Median OS for patients with CYFRA 21-1 >8 ng/mL was 3.0 months (95% CI 1.9–4.1) *versus* 17.7 months (95% CI 11.4–24.0) for patients with CYFRA 21-1 ⩽8 ng/mL, with a probability of being alive at 12 and 24 months of 10% and 8% respectively for CYFRA 21-1 ⩾8 ng/mL *versus* 54% and 23% for CYFRA 21-1 ⩽8 ng/mL [Figure 1(a)].

A CYFRA 21-1 level >8 ng/mL was correlated with lower OS at multivariate analysis (HR 1.90; 95% CI 1.24–2.93); p 0.003). Other factors correlated to OS in multivariate analysis were ECOG PS 2 (HR 3.81; 95% CI 2.39–6.08; $p < 0.001$), neutrophil to lymphocyte (N/L) ratio

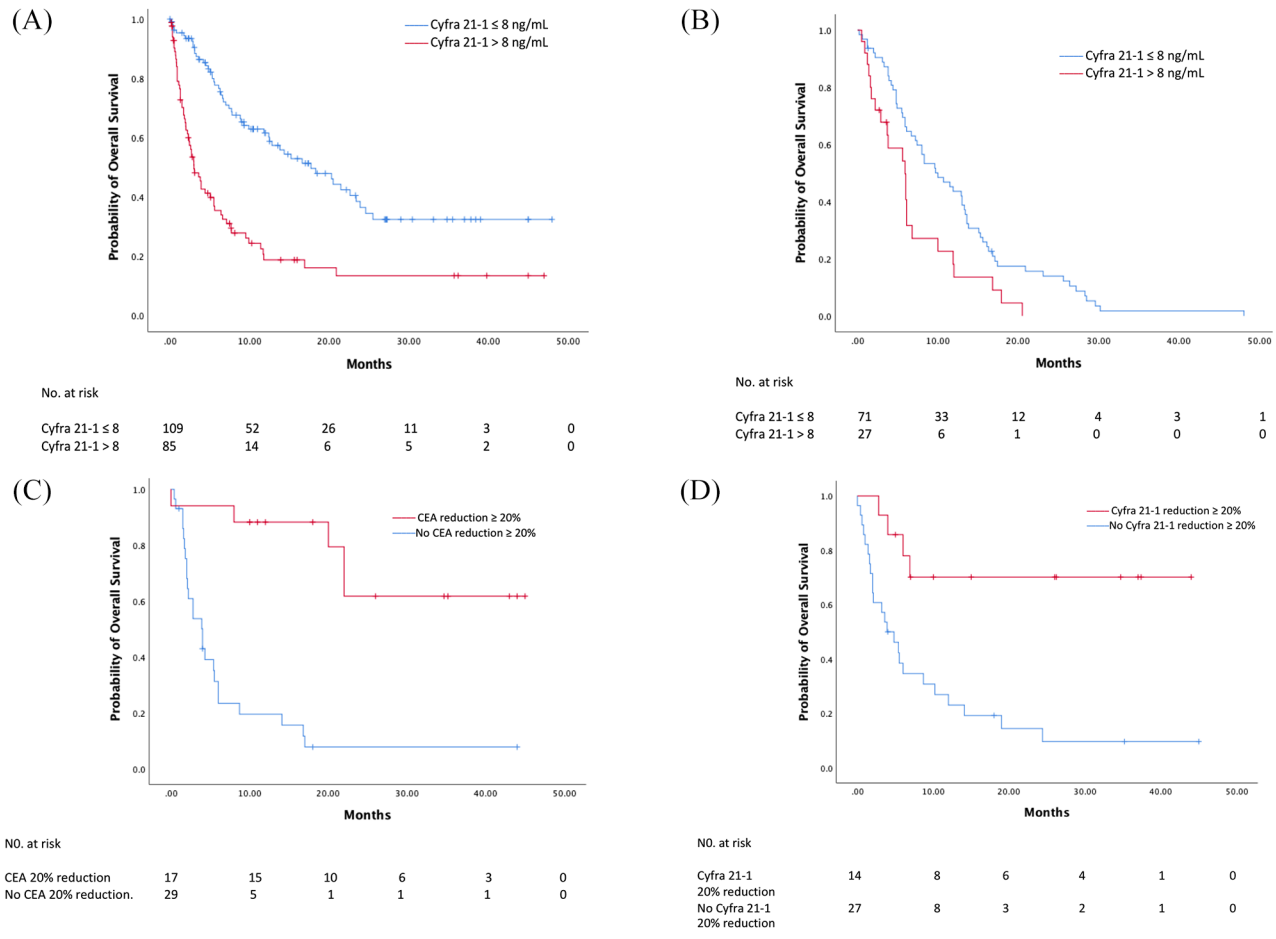

Figure 1. Overall survival (OS) according to CYFRA 21-1 in the immunotherapy pooled cohort (a) and chemotherapy cohort (b). OS according to CEA 20% reduction (c) and CYFRA 21-1 20% reduction (d).
CEA, carcinoembryonic antigen; CYFRA 21-1, cytokeratin-19 fragments.

⩾4 (HR 1.68; 95% CI 1.10–2.58; p 0.017) and CEA >8 ng/mL (HR 1.58; 95% CI 1.06–2.33; p 0.024). The final model for OS was further validated with a resampling bootstrap procedure (1000 replications) in which all statistical analyses were replicated on each bootstrapped sample, confirming the independent prognostic role of CYFRA 21-1 >ULN and ECOG PS (Table 2).

CYFRA 21-1 >8 ng/mL was also correlated with lower DCR (OR 0.43; 95% CI 0.20–0.92; p 0.03) (Table 3). C-index of the final model comprising CYFRA 21-1 >8 ng/mL, PS 2, N/L ratio ⩾4 and presence of liver metastasis was 0.728 (SE [standard error] 0.019), p <0.001 (bias corrected C-index 0.718).

Chemotherapy cohort

Clinical records of 120 patients were analysed and 22 were excluded for missing STM baseline blood levels. Clinical and pathological characteristics of 98 included patients are summarized in Table 1. All patients received first line platinum-based chemotherapy, 54.5% carboplatin and 45.5% cisplatin, in combination with gemcitabine (47.1%) pemetrexed (22.9%) and vinorelbine (28.1%).

Median OS was 8.3 months (95% CI 6.3–10.4). For patients with CYFRA 21-1 >8.0 ng/mL it was 5.9 months (95% CI 3.4–8.5) *versus* 10.0 (95% CI 6.2–13.8) for CYFRA 21-1 <8.0 ng/mL; HR 1.99 95% CI 1.21–3.27, p 0.007 [Figure 1(b)].

The final model for the immunotherapy pooled cohort was evaluated also in the chemotherapy cohort, with a C-index of 0.577 (se = 0.045), p 0.08 (bias corrected C-index 0.529).

As exploratory analysis an interaction test between immunotherapy *versus* chemotherapy and CYFRA

Table 2. Univariate and multivariate analysis for overall survival and internal validation.

	Univariate		Multivariate		Internal validation
Variable	**HR (95% CI)**	***p* value**	**HR (95% CI)**	***p* value**	**BCA HR 95% CI**
ECOG Performance Status					
0–1	1 (reference)	<0.001		<0.001	
2	4.33 (2.94–3.39)		3.81 (2.39–6.08)		**2.24–8.00**
CYFRA 21-1					
⩽8	1 (reference)	<0.001		0.003	
>8	2.89 (1.99–4.20)		1.90 (1.24–2.93)		**1.06–3.55**
Neutrophil/lymphocyte ratio					
<4	1 (reference)	<0.001		0.017	
⩾4	2.18 (1.54–3.09)		1.68 (1.10–2.58)		1.06–2.60
Liver metastasis					
No	1 (reference)	0.006		0.106	
Yes	1.85 (1.20–2.87)		1.52 (0.92–2.54)		0.87–2.81
Bone metastasis					
No	1 (reference)	0.078		0.905	
Yes	1.40 (0.96–2.02)		1.03 (0.67–1.58)		0.65–1.60
CEA					
⩽8	1 (reference)	0.027		0.024	
>8	1.49 (1.05–2.12)		1.58 (1.06–2.33)		0.89–2.32
Brain metastasis					
No	1 (reference)	0.392			
Yes	1.20 (0.79–1.84)				
Histologic subtype					
Non-squamous	1 (reference)	0.939			
Squamous	0.99 (0.67–1.45)				
Sex					
Male	1 (reference)	0.543			
Female	1.13 (0.77–1.64)				
Stage					
IIIB	1 (reference)	0.05			
IV	2.06 (1.00–4.26)		1.33 (0.63–2.83)	0.458	

(Continued)

Table 2. (Continued)

	Univariate		Multivariate		Internal validation
Variable	**HR (95% CI)**	***p* value**	**HR (95% CI)**	***p* value**	**BCA HR 95% CI**
Age					
<70	1 (reference)	0.788			
⩾70	0.95 (0.67–1.36)				
PD-L1					
<1%	1 (reference)	0.518			
⩾1%	1.21 (0.68–2.18)				

CEA, carcinoembryonic antigen; CI, confidence interval; CYFRA 21-1, cytokeratin-19 fragments; HR, hazard ratio; BCA, Bias Corrected and Accelerated.

Table 3. Univariate and multivariate analysis for disease control rate and internal validation.

	Univariate		Multivariate		Internal validation
Variable	**OR (95% CI)**	***p* value**	**OR (95% CI)**	***p* value**	**BCA OR (95% CI)**
ECOG PS					
0–1	1 (reference)	0.001		0.319	
2	0.15 (0.05–0.46)		0.54 (0.16–1.81)		0.18–1.21
CYFRA 21-1					
⩽8	1 (reference)	0.01		0.03	
>8	0.40 (0.20–0.80)		0.43 (0.20–0.92)		0.18–0.96
Neutrophil/lymphocyte ratio					
<4	1 (reference)	0.003		0.089	
⩾4	0.39 (0.20–0.73)		0.52 (0.24–1.10)		0.23–1.24
Liver metastasis					
No	1 (reference)	0.072		0.197	
Yes	0.44 (0.18–1.08)		0.51 (0.19–1.41)		0.16–1.92
Bone metastasis					
No	1 (reference)	0.005		0.003	
Yes	0.36 (0.18–0.74)		0.29 (0.13–0.65)		0.14–0.60
CEA					
⩽8	1 (reference)	0.285			
>8	0.70 (0.37–1.34)				

(Continued)

Table 3. (Continued)

	Univariate		Multivariate		Internal validation
Variable	**OR (95% CI)**	***p* value**	**OR (95% CI)**	***p* value**	**BCA OR (95% CI)**
Brain metastasis					
No	1 (reference)	0.08		0.328	
Yes	0.48 (0.21–1.09)		0.62 (0.23–1.63)		0.21–1.64
Histologic subtype					
Non-squamous	1 (reference)	0.835			
Squamous	1.08 (0.54–2.16)				
Sex					
Male	1 (reference)	0.137			
Female	1.67 (0.85–3.27)				
Stage					
IIIB	1 (reference)	0.989			
IV	0.99 (0.35–2.79)				
Age					
<70	1 (reference)	0.589			
⩾70	1.24 (0.57–2.72)				
PD-L1					
<1%	1 (reference)				
⩾1%	1.96 (0.76–5.05)	0.165			

CEA, carcinoembryonic antigen; CI, confidence interval; CYFRA 21-1, cytokeratin-19 fragments; OR, odds ratio; BCA, Bias Corrected and Accelerated.

21-1 levels above *versus* below 8 ng/mL was performed, suggesting a higher impact of CYFRA 21-1 levels on OS for ICI treated patients than for chemotherapy treated ones (HR for interaction 2.17; 95% CI 1.17–4.01; p 0.014; Supplemental Table A.3; Figure A.4).

Prognostic value of tumor markers change during therapy

Overall, 93 patients (56%) had at least one serum marker evaluation other than basal, 90 for CEA and 78 for CYFRA 21-1.

Excluding cases with baseline serum tumor markers ⩽ULN, data on serum tumor markers after the third cycle (mean 4.6 weeks from therapy start) were available in 46 and 42 cases for CEA and CYFRA 21-1 respectively.

A reduction ⩾20% after the third cycle was correlated with DCR both for CEA (OR 12.28; 95% CI 2.57–58.59; p 0.002) and for CYFRA 12-1 (OR 7.50; 95% CI 1.73–33.03; p 0.008).

Median OS was calculated from the evaluation of serum CEA after the third cycle and was Not Reached (NR) (95% CI NR–NR) for patients with a reduction of CEA blood level as compared with 4.0 (95% CI 2.1–5.9) in patients without reduction [HR 0.12; 95% CI 0.04–0.33; $p < 0.001$; Figure 1(c)]. For patients with 20% reduction in CYFRA 21-1 after the third cycle OS was NR (95% CI NR–NR) *versus* 4.0 months

(95% CI 2.0–5.0) in patients without [HR 0.19; 95% CI 0.07–0.55; p 0.002; Figure 1(d)].

Discussion

Despite the undoubted clinical progress achieved with the introduction of ICIs in the treatment of advanced NSCLC, only a minority of patients benefit from this novel and very expensive method of treatment. As things currently stand, prognostic/predictive factors allowing for the identification of patients most likely to achieve a significant benefit from immunotherapy are still lacking. Identifying easy and affordable tools to predict immunotherapy efficacy in advanced NSCLC should be considered currently a high-priority research area and one of the most relevant unmet clinical needs.

In this series, we have retrospectively evaluated the prognostic value of CEA and CYFRA 21-1 pre-therapy blood levels in 207 consecutive NSCLC patients treated with nivolumab, pembrolizumab or atezolizumab and in a historical control group treated with chemotherapy only. We have also evaluated the role of STM in treatment monitoring in patients treated with ICI.

We observed that baseline CYFRA 21-1 levels above 8 ng/mL were strongly predictive of lower disease control and shorter OS. The rapid drop of the survival curve in the first 3–6 months for the group with higher CYFRA 21-1 levels, with an extremely low median OS (2.7 for pretreated and 3.0 for first line) is particularly impressive. This finding could generate a hypothesis for a correlation with the so called hyper progressive disease.[25,26]

The effect of CYFRA 21-1 is also observed in the chemotherapy control group and this finding is consistent with previously reported evidence both in advanced NSCLC treated with chemotherapy and target therapies[27,28] and in localized NSCLC.[29,30] However, considering the impact on both the long survival tail and the initial drop of the curves on the ICI cohort (both absent in the chemotherapy cohort), the prognostic information that can be provided by CYFRA 21-1 and levels seems to be of higher value for patients treated with immunotherapy than for those treated with chemotherapy. Currently, our results do not allow us to draw a definitive conclusion as to whether elevated CYFRA levels could be considered specifically predictive of ICPIs' efficacy or more generally prognostic of a poor outcome regardless of the type of treatment administered. That said, however, CEA levels resulted significantly associated with OS for pretreated patients only (test set) but not in the first line pembrolizumab cohort (validation set), thus reducing the utility and affecting the validation of the prognostic value of this STM.

Serum CYFRA 21.1 and CEA level have been reported to be significantly higher in patients with locoregionally advanced and metastatic disease compared with those with localized disease, while CYFRA 21-1 was also correlated to total metabolic tumor volume (MTV) in a paper that addressed this issue.[31]

Papers addressing the issue of the correlation between tumor burden and the outcome of ICI reported conflicting results. A paper using fluorodeoxyglucose (*FDG*)-positron emission tomography (*PET*) MTV found a worse outcome for patients with higher MTV,[32] while other authors reported no difference using the sum of the longest diameters according to RECIST criteria.[33]

Our paper did not include a parameter such as MTV or other tumor burden measurement. However, our multivariate analysis included the stage (IIIB *versus* IV) and the presence of liver, bone and brain metastasis that are signs of disseminated disease, and confirmed the prognostic validity of STM blood levels, thus suggesting that STM are not surrogates of tumor burden but retain their independent prognostic validity.

The association between STM levels and the outcome appears to be stronger when considering patients treated in more advanced lines of treatment compared with first line. This could be due to the effect of subsequent therapies such as platinum-based chemotherapy that is frequently administered after progression to pembrolizumab, or to the lower number of patients enrolled. Another reason could be found in the choice of the cut-off (8 ng/mL) that has been chosen in the pre-treated group.

First line setting deserves further investigation as this is the setting where a predictive biomarker could make the biggest impact, considering that pembrolizumab can be administered both individually or in combination with chemotherapy. Choosing the right cut-off for CYFRA 21-1 and validating it in a different data set could help physicians provide the right therapy to the right patient.

Other studies addressed the effect of serum tumor markers on ICI outcome in NSCLC, using different

cutoffs and drawing discordant conclusions. A recent study analyzed 50 patients and reported that a pretreatment serum CYFRA 21-1 level ⩾2.2 ng/ml was correlated with a better outcome in terms of PFS,[19] while according to other authors baseline serum CEA level ⩾5 ng/ml was associated with worse Progression Free Survival.[18] Conversely, another recent paper on 70 patients, with a median follow-up of 10.7 months, reported that baseline CEA <5.0 ng/mL and CYFRA 21-1 levels <3.3 ng/mL were borderline correlated to a better OS in patients treated with nivolumab.[20]

Despite the retrospective nature of our study, it certainly has strengths such as its rigorous methodology (presence of test, validation and control cohorts together with the attempt to set an optimal STM cutoff) the relatively high number of patients enrolled, the longer follow-up, the attempt to set an optimal cutoff and the correlation with OS, which allows us to better elucidate the long term impact of CYFRA 21-1 baseline level. This can be particularly valuable for a treatment such as immunotherapy that is capable of producing long term survivors. Moreover, our multivariate model included well established prognostic factors such as N/L ratio, PD-L1 status, site of metastases ECOG PS.

Finally, data regarding the value of CEA and CYFRA 21-1 repeated measurement for disease monitoring during immunotherapy showed a significant correlation between early CEA and CYFRA 21-1 20% reduction and DCR.

A similar 20% cutoff has been shown to discriminate between responders and non-responders for chemotherapy, as shown by a recent metanalysis[16] and other recent papers, which suggests the same about ICI.[20,22] The impressive separation of the curves for STM reduction after three cycles of therapy and the long survival of those patients with reduction confirms the utility of reassessing STM blood level as an early surrogate marker of benefit for NSCLC patients treated with ICIs.

Our study has, however, several limitations. The retrospective nature of this study implies the possibility of missing clinical and pathological data, including the non-assessment of a high proportion of patients with PD-L1 expression. Moreover, a significant proportion of patients with CYFRA 21-1 above 8 ng/mL can benefit from ICI.

Conclusion

Our data supports the routine basal blood measurement of CYFRA 21-1 and CEA in patients with advanced NSCLC undergoing treatment with ICPIs, both in first-line and in second or further lines, as well as their serial reassessment during the course of the therapy.

As shown in the Kaplan–Meier plots, the early and large separation of OS curves for advanced NSCLC patients according to CYFRA 21-1 baseline levels suggests that this simple and relatively inexpensive test may provide clinicians with relevant prognostic information, in addition to clinical characteristics, that could help in selecting patients more suitable and likely to benefit from anti-cancer therapy. For example, it could be envisaged that the more aggressive course of disease in patients with high CYFRA 21-1 basal levels could require a more aggressive therapeutic strategy such as the combination of chemotherapy and immunotherapy even in cases with PD-L1 ⩾50%. Moreover, CYFRA 21-1 could also be used to stratify patients in randomized studies.

Both CEA and CYFRA 21-1 repeated blood measures during immunotherapy could help clinicians in assessing the outcome of early treatment without the need for frequent and expensive imaging procedures and, importantly, in discriminating real disease progression from pseudo-progression.

Author contributions

Data curation: Francesca Abbati, Francesco Facchinetti, Maria Massucci, Anna Squadrilli, Barbara Melotti, Francesca Formica and Sebastiano Buti

Conceptualization, formal analysis, writing – original draft: Filippo G. Dall'Olio

Funding acquisition: Andrea Ardizzoni

Supervision, writing – review and editing: Andrea Ardizzoni, Marcello Tiseo

Conflict of interest statement

Marcello Tiseo: honoraria for advisory board from BMS, MSD, Astra Zeneca, BI, Takeda; Contracted/Support Research Grant from Astra Zeneca. Andrea Ardizzoni: grants from BMS and Celgene; personal fees from BMS, MSD, Eli Lilly, Boehringer, Pfizer and Celgene. The other authors do not report any relevant conflict of interest.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This research has been funded by the no-profit organization 'AIRC – Fondazione AIRC per la Ricerca sul Cancro' (investigator grant 19026).

ORCID iD

Sebastiano Buti https://orcid.org/0000-0003-0876-0226

Supplemental material

Supplemental material for this article is available online.

References

1. Brahmer J, Reckamp KL, Baas P, *et al.* Nivolumab versus docetaxel in advanced squamous-cell non–small-cell lung cancer. *N Engl J Med* 2015; 373: 123–135.
2. Borghaei H, Paz-Ares L, Horn L, *et al.* Nivolumab versus docetaxel in advanced nonsquamous non–small-cell lung cancer. *N Engl J Med* 2015; 373: 1627–1639.
3. Herbst RS, Baas P, Kim D-W, *et al.* Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *Lancet* 2016; 387: 1540–1550.
4. Rittmeyer A, Barlesi F, Waterkamp D, *et al.* Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. *Lancet* 2017; 389: 255–265.
5. Reck M, Rodríguez-Abreu D, Robinson AG, *et al.* Pembrolizumab versus chemotherapy for PD-L1–positive non–small-cell lung cancer. *N Engl J Med* 2016; 375: 1823–1833.
6. Gandhi L, Rodríguez-Abreu D, Gadgeel S, *et al.* Pembrolizumab plus chemotherapy in metastatic non–small-cell lung cancer. *N Engl J Med* 2018; 378: 2078–2092.
7. Socinski MA, Jotte RM, Cappuzzo F, *et al.* Atezolizumab for first-line treatment of metastatic nonsquamous NSCLC. *N Engl J Med* 2018; 378: 2288–2301.
8. McLaughlin J, Han G, Schalper KA, *et al.* Quantitative assessment of the heterogeneity of PD-L1 expression in non-small-cell lung cancer. *JAMA Oncol* 2016; 2: 46–54.
9. Munari E, Zamboni G, Marconi M, *et al.* PD-L1 expression heterogeneity in non-small cell lung cancer: evaluation of small biopsies reliability. *Oncotarget* 2017; 8: 90123–90131.
10. Carbone DP, Reck M, Paz-Ares L, *et al.* First-line nivolumab in stage IV or recurrent non–small-cell lung cancer. *N Engl J Med* 2017; 376: 2415–2426.
11. Hellmann MD, Rizvi NA, Goldman JW, *et al.* Nivolumab plus ipilimumab as first-line treatment for advanced non-small-cell lung cancer (CheckMate 012): results of an open-label, phase 1, multicohort study. *Lancet Oncol* 2017; 18: 31–41.
12. Johnson DB, Frampton GM, Rioth MJ, *et al.* Targeted next generation sequencing identifies markers of response to PD-1 blockade. *Cancer Immunol Res* 2016; 4: 959–967.
13. Garassino M, Rodriguez-Abreu D, Gadgeel S, *et al.* OA04.06 Evaluation of TMB in KEYNOTE-189: pembrolizumab plus chemotherapy vs placebo plus chemotherapy for nonsquamous NSCLC. *J Thorac Oncol* 2019; 14: S216–S217.
14. Langer C, Gadgeel S, Borghaei H, *et al.* OA04.05 KEYNOTE-021: TMB and outcomes for carboplatin and pemetrexed with or without pembrolizumab for nonsquamous NSCLC. *J Thorac Oncol* 2019; 14: S216.
15. Ardizzoni A, Cafferata MA, Tiseo M, *et al.* Decline in serum carcinoembryonic antigen and cytokeratin 19 fragment during chemotherapy predicts objective response and survival in patients with advanced nonsmall cell lung cancer. *Cancer* 2006; 107: 2842–2849.
16. Holdenrieder S, Wehnl B, Hettwer K, *et al.* Carcinoembryonic antigen and cytokeratin-19 fragments for assessment of therapy response in non-small cell lung cancer: a systematic review and meta-analysis. *Br J Cancer* 2017; 116: 1037–1045.
17. Edelman MJ, Hodgson L, Rosenblatt PY, *et al.* CYFRA 21-1 as a prognostic and predictive marker in advanced non-small-cell lung cancer in a prospective trial: CALGB 150304. *J Thorac Oncol* 2012; 7: 649–654.
18. Kataoka Y, Hirano K, Narabayashi T, *et al.* Carcinoembryonic antigen as a predictive biomarker of response to nivolumab in non–small cell lung cancer. *Anticancer Res* 2018; 38: 559–563.
19. Shirasu H, Ono A, Omae K, *et al.* CYFRA 21-1 predicts the efficacy of nivolumab in patients with advanced lung adenocarcinoma. *Tumor Biol* 2018; 40: 101042831876042.

20. Dal Bello MG, Filiberti RA, Alama A, *et al.* The role of CEA, CYFRA21-1 and NSE in monitoring tumor response to nivolumab in advanced non-small cell lung cancer (NSCLC) patients. *J Transl Med* 2019; 17: 74.

21. Dall'Olio FG, Brocchi S, Massucci M, *et al.* Distinguishing between immune-related pneumonitis and disease progression in advanced non small cell lung cancer treated with PD-1 inhibitors: can serum tumor markers have a role? *Eur J Cancer* 2018; 95: 127.

22. Lang D, Horner A, Brehm E, *et al.* Early serum tumor marker dynamics predict progression-free and overall survival in single PD-1/PD-L1 inhibitor treated advanced NSCLC—A retrospective cohort study. *Lung Cancer* 2019; 134: 59–65.

23. McShane LM, Altman DG, Sauerbrei W, *et al.* REporting recommendations for tumor MARKer prognostic studies (REMARK). *Br J Cancer* 2005; 93: 387–391.

24. Facchinetti F, Veneziani M, Buti S, *et al.* Clinical and hematologic parameters address the outcomes of non-small-cell lung cancer patients treated with nivolumab. *Immunotherapy* 2018; 10: 681–694.

25. Champiat S, Dercle L, Ammari S, *et al.* Hyperprogressive disease is a new pattern of progression in cancer patients treated by anti-PD-1/PD-L1. *Clin Cancer Res* 2017; 23: 1920–1928.

26. Ferrara R, Mezquita L, Texier M, *et al.* Hyperprogressive disease in patients with advanced non–small cell lung cancer treated with PD-1/PD-L1 inhibitors or with single-agent chemotherapy. *JAMA Oncol* 2018; 4: 1543–1552.

27. Baek AR, Seo HJ, Lee JH, *et al.* Prognostic value of baseline carcinoembryonic antigen and cytokeratin 19 fragment levels in advanced non-small cell lung cancer. *Cancer Biomarkers* 2018; 22: 55–62.

28. Ono A, Takahashi T, Mori K, *et al.* Prognostic impact of serum CYFRA 21–1 in patients with advanced lung adenocarcinoma: a retrospective study. *BMC Cancer* 2013; 13: 354.

29. Muley T, Dienemann H and Ebert W. Increased CYFRA 21-1 and CEA levels are negative predictors of outcome in p-stage I NSCLC. *Anticancer Res* 2003; 23: 4085–4093.

30. Muley T, Rolny V, He Y, *et al.* The combination of the blood based tumor biomarkers cytokeratin 19 fragments (CYFRA 21-1) and carcinoembryonic antigen (CEA) as a potential predictor of benefit from adjuvant chemotherapy in early stage squamous cell carcinoma of the lung (SCC). *Lung Cancer* 2018; 120: 46–53.

31. Dogan I, Karyagar S, Karyagar SS, *et al.* Relationship between pretreatment levels of serum Cyfra 21.1, CEA and PET metabolic parameters in NSCLC. *Ann Nucl Med* 2014; 28: 829–835.

32. Seban RD, Mezquita L, Berenbaum A, *et al.* Baseline metabolic tumor burden on FDG PET/CT scans predicts outcome in advanced NSCLC patients treated with immune checkpoint inhibitors. *Eur J Nucl Med Mol Imaging*. Epub ahead of print 21 November 2019. DOI: 10.1007/s00259-019-04615-x.

33. Popat V, Lu R, Ahmed M, *et al.* Lack of association between radiographic tumor burden and efficacy of immune checkpoint inhibitors in advanced lung cancer. *Oncologist*. Epub ahead of print 31 March 2020. DOI: 10.1634/theoncologist.2019-0814.