Monitoring blood biomarkers to predict nivolumab effectiveness in NSCLC patients

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Francesco Passiglia, Antonio Galvano, Marta Castiglia, Lorena Incorvaia, Valentina Calò, Angela Listì, Salvatore Mazzarisi, Alessandro Perez, Giuseppe Gallina, Sergio Rizzo, Hector Soto Parra, Viviana Bazan and Antonio Russo

Abstract

Background: We investigated whether early dynamic changes of circulating free (cfDNA) levels as well as the neutrophil to lymphocyte ratio (NLR) could predict nivolumab effectiveness in pretreated patients with advanced non-small cell lung cancer (NSCLC).

Methods: A total of 45 patients receiving nivolumab 3 mg/kg every 2 weeks were enrolled. Patients underwent a computed tomography scan and responses were evaluated by the response evaluation criteria in solid tumors. Peripheral blood samples were obtained from the patients and the cfDNA level as well as the NLR were assessed. Time to progression (TTP) and overall survival (OS) were determined.

Results: Patients with increased cfDNA >20% at the sixth week reported significantly worse survival outcomes (median OS: 5.7 *versus* 14.2 months, $p < 0.001$; median TTP: 3.3 *versus* 10.2 months, $p < 0.001$), as well as patients with increased NLR >20% (median OS: 8.7 *versus* 14.6 months, $p = 0.035$; median TTP: 5.2 *versus* 10.3 months, $p = 0.039$). The combined increase of cfDNA and NLR >20% was associated with significantly worse survival outcomes as compared with the remained population (median OS: 5.8 *versus* 15.5 months, $p = 0.012$; median TTP: 3.2 *versus* 11.9 months, $p = 0.028$). Multivariable analysis identified three significant factors associated with worse OS: combined cfDNA/NLR increase >20% [hazard ratio (HR): 5.16; 95% confidence interval (CI), 1.09–24.29; $p = 0.038$], liver metastasis (HR: 0.44; 95% CI, 0.20–0.96; $p = 0.038$), and extra-thoracic disease (HR: 0.33; 95% CI, 0.12–0.89; $p = 0.029$).

Conclusion: An early combined increase of both cfDNA and NLR over the course of the first 6 weeks of nivolumab therapy predicted worse survival in pretreated patients with advanced NSCLC, suggesting a potential role in the real-time monitoring of immunotherapy resistance.

Keywords: blood-based biomarkers, Circulating free DNA (cfDNA), immunotherapy, neutrophil to lymphocyte ratio (NLR), non-small cell lung cancer (NSCLC).

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Correspondence to:
Antonio Russo
Department of Oncology, A.O.U.P. 'P. Giaccone' University Hospital, 2013 ESMO Designated Centres of Integrated Oncology and Palliative Care, Via del Vespro 129, 90127 Palermo, Italy
antonio.russo@usa.net
Francesco Passiglia
Antonio Galvano
Marta Castiglia
Lorena Incorvaia
Valentina Calò
Angela Listì
Alessandro Perez
Giuseppe Gallina
Sergio Rizzo
Viviana Bazan
Department of Surgical, Oncological and Stomatological Disciplines, University of Palermo, Palermo, Italy
Salvatore Mazzarisi
Hector Soto Parra
Medical Oncology Unit, AOU Policlinico Vittorio Emanuele, Catania, Italy

Introduction

In the last few years we have witnessed a great step forward in the treatment of advanced non-small cell lung cancer (NSCLC), thanks to the advent of immune-checkpoint inhibitors (ICIs) in clinical practice.[1] Overall, four randomized phase III trials[2–5] have consistently demonstrated that single agent programmed cell death (PD)-1 /programmed death ligand (PD-L)1 inhibitors significantly improved overall survival (OS) and quality of life (QoL) over docetaxel, representing the new standard of care for NSCLC patients who experienced disease progression after platinum combinations. Pembrolizumab showed a significant survival benefit as compared with first-line platinum-chemotherapy in nononcogene-addicted NSCLC patients with a PD-L1 tumor proportion score of 50% or greater.[6] More recently pembrolizumab was also approved in combination with first-line carboplatin-pemetrexed based on an improved OS observed in the phase III Keynote 189 trial, regardless of tumor PD-L1 expression status. To date we have three ICIs, nivolumab, pembrolizumab and atezolizumab

approved in pretreated patients, with pembrolizumab recommended also as a first-line option both as a single agent and in combination with platinum chemotherapy.[7] Data emerging from both randomized trials and real-life experiences[8,9] suggested that although immunotherapy worked very well in a significant subgroup of patients, about 50% of them did not gain any benefit from ICIs. Worth mentioning is also the recent evidence that 10–15% of pretreated NSCLC patients developed 'hyperprogression' to immunotherapy,[10,11] defined as a rapid and dramatic increase of tumor burden during ICI administration. Thus, identifying predictive biomarkers of clinical response /resistance to ICIs remains a crucial issue for translational research. In addition to tumor PD-L1 expression, several other clinical and biological parameters have been investigated as biomarkers for clinical use. A *post hoc* exploratory analysis of the checkmate 057 trial showed that nivolumab-treated patients with lower or no tumor PD-L1 expression and poorer prognostic factors, including Eastern Cooperative Oncology Group (ECOG) performance status (PS) = 1, time since last treatment less than 3 months, and progression of disease as best response to prior therapy, were at higher risk of early death during drug administration.[12] Data emerging from published studies and meta-analyses demonstrated that epidermal growth factor receptor (EGFR) activating mutations,[13] inactivation of *LKB1*/*STK11* tumor suppressor gene,[14] low tumor mutational burden (TMB)[15] and low tumor-infiltrating lymphocytes (TILs)[16] were associated with poor responses to immunotherapy. Furthermore, a defective or absent pre-existing immune response was associated with innate/acquired resistance to ICIs,[17,18] but the predictive role of immune-gene signatures is still under validation in clinical trials. All these biomarker analyses require high quality tumor tissue that in many cases is not available for patients with metastatic disease who receive ICIs in later lines of treatment. For this reason, several efforts are currently ongoing to identify easily detectable peripheral blood parameters which could guide clinical selection and real-time monitoring of lung cancer patients receiving immunotherapy.

Multiple studies and meta-analyses revealed that elevated neutrophil to lymphocyte ratio (NLR) is associated with a poor prognosis in patients with lung cancer.[19–21] Recently other studies investigated the association between pretreatment NLR and survival in patients with advanced NSCLC, overall showing controversial results.[22–28] Circulating tumor DNA (ctDNA) is emerging as another promising biomarker, since monitoring its level changes in the peripheral blood of ICI-treated patients with lung cancer provide a real-time snapshot of active tumor cell death as well as a reliable measure of overall tumor burden.[29] In the present study we investigated whether combined and dynamic assessment of cell-free DNA (cfDNA) and NLR variations over the course of the first 6 weeks of nivolumab therapy may predict nivolumab effectiveness in terms of time to progression (TTP) and overall survival (OS) in pretreated patients with advanced NSCLC.

Materials and methods

Patients

Patients were eligible if they were aged ⩾18 years, had histologically or cytologically confirmed diagnosis of NSCLC, stage IIIB–C/IV (according to version 8 of the International Association for the Study of Lung Cancer TNM Staging System), EGFR/Anaplastic lymphoma kinase (ALK): wildtype, ECOG-PS < 3, disease progression or recurrence after receiving at least one prior systemic therapy for advanced/metastatic disease, with blood cell count and blood samples available.

Patients were excluded if they had autoimmune disease, symptomatic interstitial lung disease, systemic immunosuppression, prior treatment with immune-stimulatory antitumor agents including checkpoint inhibitors. Patients with brain metastases were eligible if they had received prior locoregional treatment and were stable at the time of eligibility. Tumor PD-L1 status was not required.

The study was conducted in accordance with the International Conference on Harmonization Guidelines on Good Clinical Practice and the Declaration of Helsinki. The trial protocol was previously approved by the Independent Ethic Committee at University of Palermo (ethics approval number: 0006981) and all the patients provided a written informed consent before enrollment.

Study design and treatment

From September 2015 to January 2018 eligible patients were included and received nivolumab 3 mg/kg intravenously every 2 weeks until disease progression or unacceptable toxicity.

We retrospectively collected clinical data and routine blood tests from patient charts and medical records at two university hospitals.

Blood tests were obtained within 1 week prior to the first (baseline) and fourth infusion of nivolumab (sixth week) and included the white blood cell count with lymphocyte and neutrophil counts, from which the NLR was deduced.

Peripheral blood samples were collected from patients included in the study the day of the drug administration at baseline and at 6 weeks during the course of therapy. Each blood sample was immediately processed for plasma collection, according to a simple and standardized protocol, and stored frozen as 500 ml aliquots at −80°C. The cfDNA isolation was performed with the QIAamp Circulating Nucleic Acid Kit (Qiagen, 20100 Milan, Italy) starting from 2 ml of plasma according to the manufacture's instruction. The quantification of cfDNA (ng/ml plasma) was performed by qubit dsDNA HS assay (Thermo Fisher Scientific, 20090 Rodano, Milan, Italy) and confirmed by qPCR on a 7900HT instrument (Applied Biosystems, 20900 Monza and Brianza - Italy) evaluating a 115 bp fragment of Arthrobacter luteus (ALU) repeats.

Radiological evaluation of treatment efficacy by computed tomography (CT) scan was performed at week 12 and every 12 weeks of therapy, thereafter until disease progression and responses were evaluated by the response evaluation criteria in solid tumors (RECIST) version 1.1.

Statistical analysis

The cfDNA plasma level was calculated in NSCLC patients at baseline and at the sixth week during the course of therapy with nivolumab, while the NLR was calculated from blood tests obtained from each patient at the same time points. Both median cfDNA and NLR were calculated as the 'middle number' separating the higher half from the lower half of a data sample represented by the sorted list of values derived from the patients included in the study. Median cfDNA and NLR increase were calculated as the 'middle value' of a sorted list including the differences between baseline and the 6-week measurements in each patient. A 20% increase from baseline was detected as the median increase for both cfDNA and NLR values and was used as the cut-off point for survival analysis.

The Mann–Whitney U test was used for intergroup comparisons of two independent samples, while the Fisher's exact test was used for categorical values. A paired Wilcoxon test was used to compare the median cfDNA plasma levels as well as median NLR before and after four cycles of therapy with nivolumab.

Survival analysis on the basis of the median cut-off values was performed using the Kaplan–Meier method, providing median and p values, with the use of the log-rank test for comparisons. Univariate and multivariate analyses were performed using the Cox proportional hazards and logistic regression models. All pretreatment parameters found to have a p value <0.05 at univariate analysis were included as covariates in the multivariable model.

Median TTP was defined as the time between the date of inclusion and the date of disease progression determined by RECIST version 1.1. Median OS was defined as the time between the date of inclusion and the date of death. A p value < 0.05 was used as threshold for statistical significance. All the statistical analyses were performed using SPSS statistics software, version 20 (IBM, Armonk, NY, USA).

Results

Patients' characteristics

From September 2015 to January 2018 a total of 45 patients were considered eligible and were included in the study. Clinical characteristics of the patients are summarized in Table 1. The median age was 66 years (range 51–80 years); the majority of patients were men (71.1%), current or former smokers (84.4%) and exhibited an ECOG PS < 2 (82.2%). The bone was the most common metastatic site (44.4%) followed by liver (37.8%), adrenal gland (28.9%) and central nervous system (CNS; 15.6%). Patients received a median of 9 doses of nivolumab (range: 1–57), with a median follow up of 9.1 months (range: 0.1–29.7 months).

Among 45 patients evaluable for cfDNA analysis at baseline, the median cfDNA level was 0.43 ng/ml, thus not significantly higher than that observed in 36 patients evaluated after four cycles of therapy with nivolumab (0.42 ng/ml, $p = 0.81$). The median NLR at baseline was 3.31, thus very similar to that observed after four cycles of nivolumab therapy (NLR: 3.2, $p = 0.89$).

Table 1. Baseline patient characteristics.

Characteristic	Total ($n = 45$)	%
Median age (years - range)	66 (51–80)	
Sex		
Male	32	71.1
Female	13	28.9
Histology		
Adenocarcinoma	25	55.5
Squamous cell carcinoma	20	45.5
Smoking history		
Never	7	15.6
Current/former	38	84.4
Performance status		
<2	37	82.2
$\geqslant 2$	8	17.8
Stage IV subgroup		
M1a	11	24.4
M1b–c	34	75.5
Metastatic sites		
Bone	20	44.4
Liver	17	37.8
Adrenal	13	28.9
Brain	7	15.6
Prior line of therapy		
<2	22	48.9
$\geqslant 2$	23	51.1
Median NLR		
Low (<3.3)	21	46.6
High (>3.3)	24	53.4
Median cfDNA		
Low (<0.43 ng/ml)	26	57.8
High (>0.43 ng/ml)	19	42.2

cfDNA: circulating free DNA; NLR, neutrophil to lymphocyte ratio.

Pretreatment parameters and patient survival

At the time of survival analysis (median follow up of 15.3 months, range: 2–39 months), disease progression occurred in 37 patients, while 31 patients died because of tumor progression, and 14 patients were still alive at the time of data analysis. Median TTP was 5.6 months and median OS was 8.8 months in the overall population.

To identify potential baseline biomarkers associated with nivolumab effectiveness we examined clinical-pathological characteristics, blood parameters and cfDNA levels before treatment initiation. Among clinical characteristics, ECOG-PS > 2 (OS, $p = 0.032$; TTP, $p = 0.038$), presence of extra-thoracic (M1b-c) disease (OS, $p = 0.001$; TTP, $p = 0.004$) and liver metastasis (OS, $p = 0.001$; TTP, $p = 0.031$) were significantly associated with worse survival outcomes in the overall population.

Elevated pretreatment cfDNA level was associated with inferior OS (7.2 *versus* 13.5 months, $p = 0.04$) while high NLR predicted inferior TTP (4.5 *versus* 9.7 months, $p = 0.006$) but not significant OS differences. Patients with elevated pretreatment cfDNA levels and NLR values showed a not significant trend toward worse survival outcomes as compared to patients reporting one or none of such elevated parameters (median OS: 9.4 *versus* 8.1 *versus* 9.6 *versus* 16.3 months, $p = 0.088$; median TTP: 6.2 *versus* 4.8 *versus* 7 *versus* 11.4 $p = 0.22$; Table 2).

cfDNA/NLR dynamic changes and patient survival

Among 36 out of 45 patients evaluable for cfDNA or NLR analysis after four cycles of therapy with nivolumab, a 20% median cfDNA and NLR increase has been detected and was used as the cut-off point for survival analysis.

Patients with an increased cfDNA level >20% at the sixth week reported significantly worse OS and TTP as compared with other patients (median OS: 5.7 *versus* 14.2 months, $p < 0.001$; median TTP: 3.3 *versus* 10.2 months, $p < 0.001$; Figure 1 and Table 2).

Patients with increased NLR > 20% at the sixth week showed significantly worse median OS and TTP as compared with the remaining population (median OS: 8.7 *versus* 14.6 months, $p = 0.035$; median TTP: 5.2 *versus* 10.3 months, $p = 0.039$; Figure 2 and Table 2).

The combined increase >20% of both cfDNA and NLR values between baseline and the sixth week of nivolumab therapy was associated with significantly worse survival outcomes as compared with patients with no increase of both such parameters (median OS: 5.8 *versus* 15.5 months, $p = 0.012$; median TTP: 3.2 *versus* 11.9 months, $p = 0.028$; Figure 3 and Table 2).

Multivariate analysis for survival outcomes

Multivariable Cox proportional regression analysis was performed to assess whether cfDNA or NLR increases over the course of the first 6 weeks of therapy were independent factors related to nivolumab effectiveness in terms of TTP and OS. All pretreatment parameters found to have a p value <0.05 at univariate analysis were included as covariates in the multivariable model.

Multivariable analysis identified three significant factors associated with a worse OS: combined cfDNA/NLR increase >20% [hazard ratio (HR): 5.16; 95% confidence interval (CI), 1.09–24.29; $p = 0.038$], liver metastasis (HR: 0.44; 95% CI, 0.20–0.96; $p = 0.038$), extra-thoracic disease (HR: 0.33; 95% CI, 0.12–0.89; $p = 0.029$; Table 3).

Discussion

This study represents a retrospective analysis with nivolumab in patients with previously treated, EGFR/ALK wildtype, advanced NSCLC. The results of this work demonstrated that an early combined increase of both cfDNA and NLR over the course of the first 6 weeks of nivolumab therapy is associated with worse survival outcomes, suggesting a potential role in the realtime monitoring of immunotherapy resistance. In line with other evidences,[30–32] the presence of extra-thoracic disease as well as liver metastases also remain independent predictors of poor survival in our series of nivolumab-treated patients with NSCLC.

Interest in circulating biomarkers associated with immunotherapy efficacy is rapidly growing, with many potential candidates, such as soluble PD-L1,[33] blood-based TMB,[34] serum chemokines/cytokines,[35] ctDNA,[29,36] microRNA, and circulating

Table 2. Univariate analysis for survival outcomes.

Parameter	OS		TTP	
	Median, months (95% CI)	**Log-rank *p* value**	**Median, months (95% CI)**	**Log-rank *p* value**
cfDNA/NLR increase (>20% *versus* <20%)	5.8 (4.1–7.5) 15.4 (11.3–19.6)	$p = 0.012$	3.2 (1.7–4.6) 11.9 (7.7–16.1)	$p = 0.028$
cfDNA increase (>20% *versus* <20%)	5.7 (3.1–8.3) 14.2 (10.8–17.7)	$p < 0.001$	3.3 (1.8–4.8) 10.2 (6.9–13.5)	$p < 0.001$
NLR increase (>20% *versus* <20%)	8.7 (5.7–11.8) 14.6 (11.3–17.8)	$p = 0.035$	5.2 (2.3–8.2) 10.3 (10.3–16.4)	$p = 0.039$
NLR baseline (>3.3 *versus* <3.3)	8.6 (5.5–11.8) 13 (9.6–13.3)	$p = 0.084$	4.5 (2.6–6.4) 9.8 (6.3–13.1)	$p = 0.006$
cfDNA baseline (>0.43 *versus* <0.43)	7.2 (4.4–10.04) 13.5 (10.1–17)	$p = 0.033$	4.7 (1.9–7.4) 8.9 (6.02–11.8)	$p = 0.069$
cfDNA/NLR baseline (high *versus* low)	9.4 (4.6–14.1) 16.2 (12.5–20.06)	$p = 0.088$	6.2 (0.14–12.25) 11.4 (7.6–15.3)	$p = 0.22$
Age (years) (>70 *versus* <70)	12.2 (6.8–17.6) 9.5 (6.7–12.4)	$p = 0.273$	10 (5.6–14.4) 5.5 (3.6–7.3)	$p = 0.064$
Sex (male *versus* female)	10.07 (7.05–13.08) 11.1 (6.01–16.08)	$p = 0.557$	7.1 (4.5–9.7) 6.7 (3.4–10.09)	$p = 0.8$
ECOG PS (2 *versus* 0–1)	5.3 (2.4–8.3) 11.9 (8.8–14.9)	$p = 0.016$	3 (1.7–4.2) 8 (5.5–10.3)	$p = 0.038$
Smoking habits (current/former *versus* never)	9.8 (7.1–12.5) 14.5 (6.9–22.1)	$p = 0.317$	6.03 (4.1–7.9) 12.6 (6.3–18.8)	$p = 0.088$
Histology (squamous *versus* nonsquamous)	7.6 (3.7–11.5) 11.6 (8.5–14.8)	$p = 0.182$	5.7 (2.6–8.8) 7.5 (5.03–10.1)	$p = 0.49$
Stage IV subgroup (M1b–c *versus* M1a)	7.9 (5.4–10.3) 19.3 (15.04–23.7)	$p = 0.001$	5.4 (3.2–7.5) 12.2 (8.8–15.6)	$p = 0.017$
Previous lines (>1 *versus* 1)	9.7 (6.1–13.4) 11.06 (7.3–14.8)	$p = 0.62$	6.5 (3.9–9) 7.6 (4.4–10.8)	$p = 0.63$
Brain metastasis (yes *versus* no)	9.5 (2.8–16.2) 10.6 (7.7–13.5)	$p = 0.8$	5.2 (1.5–9) 7.3 (4.9–9.6)	$p = 0.68$
Liver metastasis (yes *versus* no)	5.1 (2.5–7.7) 13.2 (10.04–16.5)	$p = 0.001$	3.9 (2.2–5.6) 8.6 (5.8–11.4)	$p = 0.018$
Adrenal metastasis (yes *versus* no)	7.2 (4.04–10.6) 11.8 (8.5–15.2)	$p = 0.09$	3.08 (1.8–4.3) 8.5 (5.9–11.09)	$p = 0.056$
Bone metastasis (yes *versus* no)	8.7 (5.6–11.8) 12.2 (8.8–16.1)	$p = 0.23$	6.3 (3–9.5) 7.6 (5.1–10.2)	$p = 0.57$

CI, confidence interval; cfDNA, circulating free DNA; ECOG-PS, Eastern Cooperative Oncology Group performance status; NLR, neutrophil to lymphocyte ratio; OS, overall survival; TTP, time to progression.

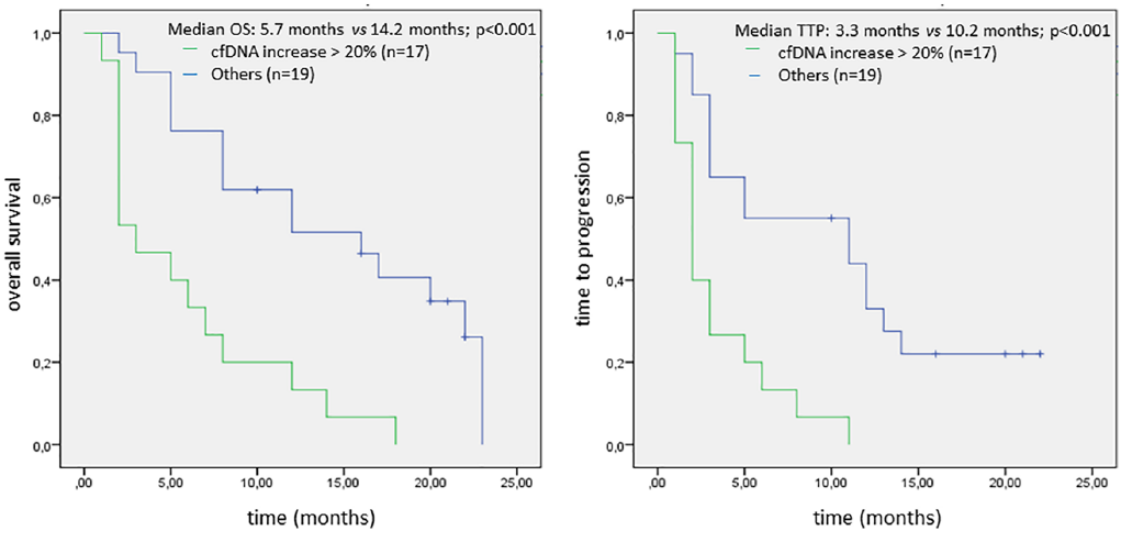

Figure 1. Kaplan–Meier analysis of OS and TTP in NSCLC patients according to the cfDNA increase.
cfDNA, circulating free DNA; NSCLC, non-small cell lung cancer; OS, overall survival; TTP, time to progression.

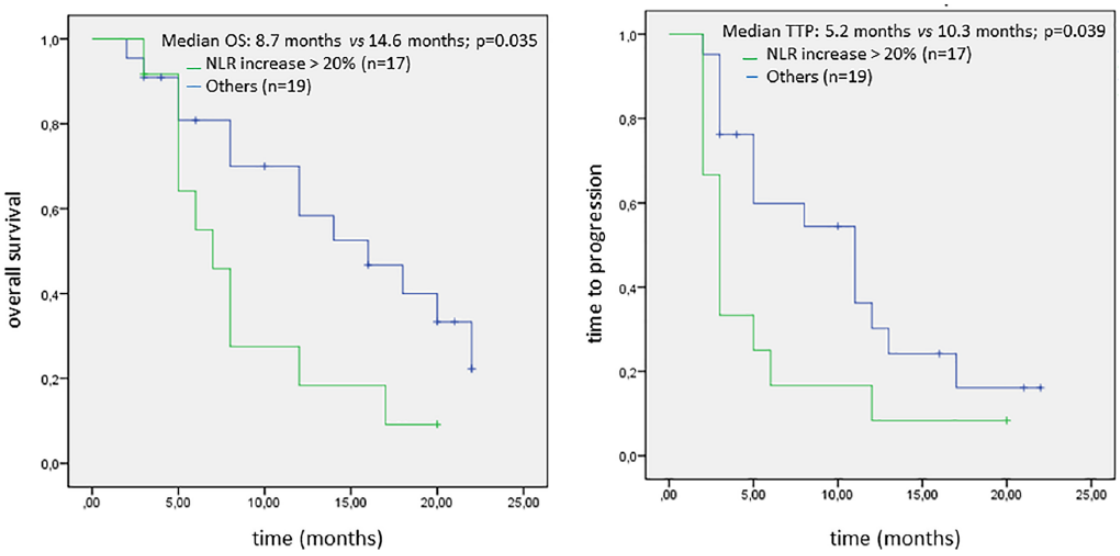

Figure 2. Kaplan–Meier analysis of OS and TTP in NSCLC patients according to the NLR increase.
NLR, neutrophil to lymphocyte ratio; NSCLC, non-small cell lung cancer; OS, overall survival; TTP, time to progression.

immune cell subsets,[37] currently under investigation in clinical studies.

In this scenario the NLR, a reliable index of systemic inflammation, represents an easy and accessible biomarker with potential application in the clinical context. Although its prognostic role in lung cancer is well established, the clinical ability of NLR in predicting ICI efficacy remains far from clear. The majority of available evidence suggests that high pretreatment NLR is associated with poor response and survival in advanced NSCLC patients treated with ICIs.[23–26] Conversely other trials did not find any significant correlation between pretreatment NLR and the clinical response to nivolumab, revealing that only a lower value of NLR at the sixth week was significantly associated with better patient survival.[27,28] Overall these studies have several limitations, including retrospective design, small number of patients, different drugs as well as different NLR cut-off points, thus questioning the reliability of this biomarker for clinical practice. Other groups included pretreatment NLR values

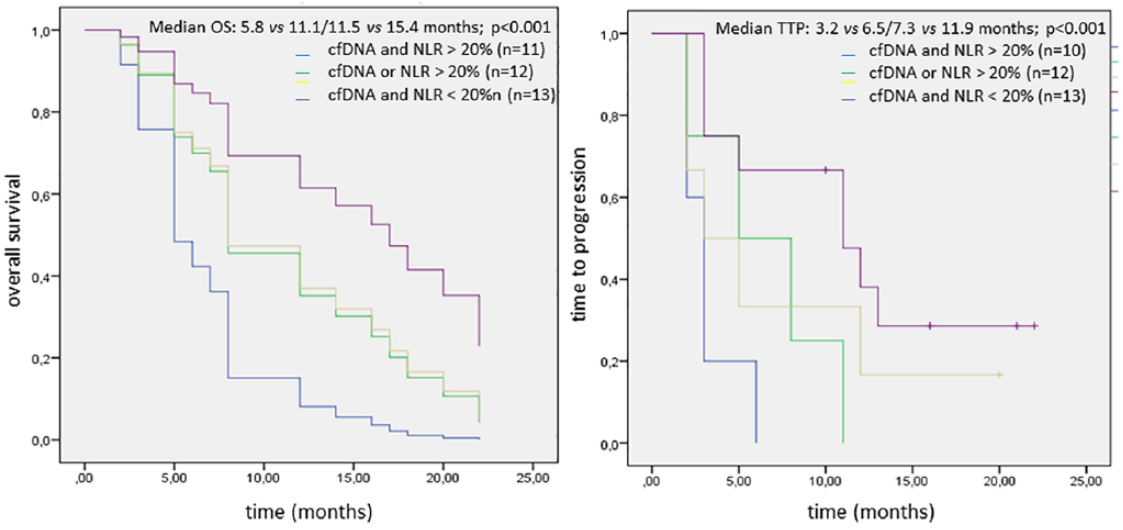

Figure 3. Kaplan–Meier analysis of OS and TTP in NSCLC patients according to the combined cfDNA/NLR increase.
cfDNA, circulating free DNA; NLR, neutrophil to lymphocyte ratio; NSCLC, non-small cell lung cancer; OS, overall survival; TTP, time to progression.

Table 3. Multivariate analysis for survival outcomes.

Parameter	OS		TTP	
	HR (95% CI);	*p* value	HR (95% CI)	*p* value
cfDNA/NLR increase (>20% *versus* <20%)	5.16 (1.09–24.29)	$p = 0.038$	0.61 (0.41–0.9)	$p = 0.013$
cfDNA increase (>20% *versus* <20%)	0.95 (0.24–3.76)	$p = 0.94$	2.37 (0.86–6.52)	$p = 0.09$
NLR increase (>20% *versus* <20%)	1.90 (0.71–5.11)	$P = 0.202$	1.89 (0.83–4.3)	$p = 0.13$
NLR baseline (low *versus* high)	—	—	0.56 (0.24–1.25)	$p = 0.16$
cfDNA baseline (low *versus* high)	0.63 (0.29–1.36)	$p = 0.24$	—	—
ECOG PS (<2 *versus* >2)	0.51 (0.21–1.24)	$p = 0.140$	0.48 (0.2–1.17)	$p = 0.11$
Stage IV subgroup (M1a *versus* M1b–c)	0.33 (0.12–0.89)	$p = 0.029$	0.43 (0.18–1.02)	$p = 0.057$
Liver metastasis (no *versus* yes)	0.44 (0.20–0.96)	$p = 0.038$	0.60 (0.26–1.39)	$p = 0.24$

CI, confidence interval; cfDNA, circulating free DNA; ECOG-PS, Eastern Cooperative Oncology Group performance status; HR, hazard ratio; NLR, neutrophil to lymphocyte ratio; OS, overall survival; TTP, time to progression.

along with other clinical-pathological features in the context of more complex prognostic scores aiming at identifying patients unlikely to benefit from immunotherapy.[38] Considering that the systemic balance between neutrophil-related inflammation and lymphocyte-mediated anti-tumor immunity may influence clinical response to immunotherapy, it is plausible that the

real-time monitoring of dynamic variations in the relative proportions of circulating immune cells under ICI therapy could more accurately reflect the therapeutic efficacy of these agents compared with baseline evaluation.

As mentioned before, the cfDNA level in the peripheral blood of patients with NSCLC provides a real-time snapshot of active tumor cell death as well as an indirect measure of overall tumor burden, thus emerging as a reliable surrogate of tumor response to immunotherapy. Recent evidence has shown that the serial monitoring of ctDNA level changes predicted tumor response and survival outcomes in patients with advanced NSCLC receiving ICI therapy.[29,36] Another paper[39] demonstrated that a higher tumor burden may be associated with a blunted CD8+ T-cell immune response, suggesting that a clinical response to PD-1 blockade is correlated with reinvigoration of exhausted CD8+ T-cells, which is inversely associated with a pretreatment tumor burden, thus providing a biological explanation for our findings.

The complementary effects of these two different approaches suggest an intriguing potential for the combined use of NLR and cfDNA in clinical practice. Indeed, the deterioration of tumor-associated inflammatory response reflected by an NLR increase along with the cfDNA level increment produced by tumor growth predicted a poor benefit to immunotherapy in pretreated patients with advanced NSCLC. Monitoring an individual patient's immune repertoire in the peripheral blood provides insights into their clinical response to immunotherapies. Thus, the early assessment of such biomarkers during the course of ICI therapy could play a critical role in treatment decision, especially when the synchronous radiological evaluation is difficult or equivocal.

This study has some limitations, including the retrospective design, the small number of patients, and the heterogeneity of clinical-pathological characteristics (e.g. high percentage of men, smokers, and PS < 2) which may have produced longer survival compared with those reported in randomized trials. Different to circulating tumor-specific DNA, cfDNA may also include nontumor DNA that is elevated as a result of cancer-related systemic inflammation, thus leading to potential detection bias in our cohort. Nevertheless, we performed a thorough evaluation of a real-world series providing preliminary evidence on the potential role of a circulating biomarker and generating a study hypothesis which should be adequately addressed by prospective trials in order to identify the most appropriate cut-offs for clinical use.

In conclusion, the results of this study showed that the early combined increase of both cfDNA and NLR over the course of the first 6 weeks of nivolumab therapy predict worse survival outcomes in pretreated patients with advanced NSCLC, suggesting a potential role in the real-time monitoring of immunotherapy resistance.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

ORCID iD

Antonio Russo https://orcid.org/0000-0002-4370-2008

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