RESEARCH ARTICLE

Neutrophil-to-lymphocyte ratio evolution is an independent predictor of early progression of second-line nivolumab-treated patients with advanced non-small-cell lung cancers

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Abstract

Introduction

Although second-line immunotherapy obtained better outcomes than chemotherapy for patients with advanced non-small–cell lung cancers (NSCLCs), it is expensive and only a minority of patients seem to benefit, based on early tumor progression post-immunotherapy. Notable host inflammation, characterized by biomarkers (e.g. neutrophil-to-lymphocyte ratio (NLR])), prolongs overall survival (OS) of surgery-, chemotherapy- and immunotherapy-treated patients. To our knowledge, no previous studies used biomarker evolution to analyze the immunotherapy impact on host inflammation. Immunotherapy mainly exerts its activity by lymphocyte reactivation.

Methods

This retrospective study was conducted on patients, selected by their progression status just before their 4th nivolumab injection, and treated at Bordeaux and Limoges University Hospitals. A comparative group of at least 1-year responders was also selected. Clinical parameters and hematological data just before the 1st (baseline) and 4th nivolumab infusions were collected to calculate the NLR change (ΔNLR) between those two infusions. The combined impact of the different known prognostic factors was also analyzed with multivariable analyses.

Results

Fifty-nine patients were included. The 29 early progressors had significantly more frequent ΔNLR > 1 ($p = 0.0007$), OR 18.08 [95% CI 2.96–246.24] with progressive disease as best response to prior treatment line ($p = 0.0014$). ΔNLR < 1 prolonged OS (HR 0.001 [0.0007–0.18], $p = 0.001$); as did a partial response to prior line of systemic treatment (HR 0.14 [0.03—0.56], $p = 0.005$).

Conclusion

Based on selected early progressors given second-line immunotherapy for advanced NSCLC, progression as best response to prior treatment and $\Delta NLR > 1$ characterized the early progressors and shortened OS after starting nivolumab. This phenomenon questions nivolumab utility in patients with a major host neutrophil inflammation.

1. Introduction

Nivolumab was the first immunotherapy approved by the Food and Drug Administration (FDA) for advanced non-small cell lung cancer (NSCLC) second-line therapy. In France, it was given as compassionate therapy before being approved by French health authorities. Nivolumab, compared to docetaxel, has prolonged overall survival (OS) of patients with squamous [1] and non-squamous NSCLCs [2]. However, in those studies, response rates were only 42% and 19%, respectively. Those responses mean that entire population did not benefit, making it critical to identify biomarkers of patients likely to respond. Nivolumab inhibits programmed cell-death protein-1 (PD-1)–mediated signaling by blocking its ligand (PD-L1))from binding to it [3], thereby preventing reactivation of cytotoxic activity [4] and expansion of clonal T cells recognizing tumor-specific antigens [5].

However, the chronic inflammation induced by tumor development also affects the tumor's growth, dissemination and immunoresistance [6]. Research has focused on immunological biomarkers that might identify and follow the equilibrium between pro-tumor and anti-tumor immunotherapy-caused inflammation. The neutrophil-to-lymphocyte ratio (NLR) is defined as the absolute neutrophil count (ANC) divided by the absolute lymphocyte count (ALC). Although many studies showed its interest, their results diverged. Some underlined the impact of NLR > 3.6 [7] or > 5 [8] just before the 1st immunotherapy infusion (baseline) on OS and progression-free survival (PFS). Others found no NLR difference from baseline to after 6 weeks of treatment [9,10]. However, because a single ratio only catches a frozen glimpse, it is difficult to extrapolate it to the immune system's perpetual movement. An early NLR decline, between the 1st and 3rd nivolumab infusions, for metastatic renal cell carcinoma patients was associated with better outcomes [11]. A study on 19 highly heterogenous NSCLC patients [12] underlined the influence of an NLR decrease on the time to treatment failure.

In this novel study, by monitoring the NLR evolution between the 1st and 4th nivolumab infusions, we aimed to determine whether NSCLC patients' inflammation-biomarker evolutions impacted immunotherapy efficacy.

2. Methods

2.1 Patients and data collection

This multicenter retrospective study included 59 patients over 18 years old, receiving second- or third-line nivolumab (3 mg/kg intravenously every 2 weeks), after one or more prior chemotherapies, between June 2015 and April 2018 at Limoges and Bordeaux University Hospitals. Previous studies [1,2] assessed the first tumor response at week 9, after 4 injections. Early progressors were defined by a progression at this first evabuation, according to to the Response Evaluation Criteria In Solid Tumors guidelines for immunotherapeutics (iRECIST version 1.1) [13]. A control group of long-term responders, defined by radiologic response or stabilization under immunotherapy lasting at least 1 year, was also

selected. Patients were excluded when [1] they had received first-line immunotherapy, [2] died before the 2nd nivolumab infusion, [3] had a concomitant infection involving an immunodeficiency or autoimmune disorder, [4] were participating in another clinical trial or [5] were under guardian or trusteeship. Electronic medical records and pharmacy databases were screened to obtain patients' specific information. Data collected included: demographics; smoking history; histology; endothelial growth factor-receptor (*EGFR*), anaplastic lymphoma kinase (*ALK*), transmembrane tyrosine-kinase receptor (*ROS1*) and Kirsten rat-sarcoma viral oncogene (*KRAS*) gene mutations, and PD-L1 status, when available; metastatic sites at initial diagnosis; description of previous treatments (numbers of cycles, time under treatment, best response to previous treatment[s]) number of nivolumab infusions received; response status; date of progression (or last follow-up) as determined by radiology reports; and date of death or last follow-up. Hematological and biochemistry parameters of interest (absolute leukocyte (ALC), neutrophil (ANC) and platelet (APC) counts, albumin (ALB) concentrations enabling calculation of NLR [8], ΔNLR, ΔANC, ΔALC, platelet-to-lymphocyte ratio (PLR) [7], lactate dehydrogenase (LDH), C-reactive protein (CRP) at the 1st and 4th infusions, the advanced lung-cancer inflammation index (ALI; (body mass index × albumin)/NLR [13] and the Lung Immune Prognostic Index (LIPI) [14].

As we compared the clinical and biological characteristics of early progressors and long responders, we studied which component had an impact on the response duration to nivolumab. Retained cut-offs were 6 Giga/L for neutrophils (upper limit of normal, ULN), NLR = 5 [7,8], and PLR = 169 and 262 [7]. An exploratory analysis evaluated the impact of ΔNLR on OS, by dividing the population according to a cut-off value of 1 chosen because the NLR standard deviation (SD) was 0.8. The median ΔANC and ΔALC were used as the dividing thresholds. OS was defined as the number of months between the 1st nivolumab infusion and death or the last follow-up.

The Ethics Committee of the Limoges University Hospital approved this study (no. 285-2018-51) and informed consent was not required because of the retrospective character of the study.

2.2 Statistical analyses

All collected data were analyzed using Statview software (SAS Institute, Inc., Cary, NC) and R software. Quantitative results are expressed as median [range] or mean ± SD and qualitative results as *n* (%) Nominal variables were compared between groups using the chi-square or Fisher's exact test, as appropriate. Means were compared with the non-parametric Mann–Whitney *U*-test for continuous variables. Univariate analyses identified variables associated with therapeutic response ($p \leq 0.2$) that were then entered into multivariate logistic-regression models. The Kaplan–Meier method was applied to evaluate the OS probability. Median OS rates were compared with the non-parametric log-rank test. Multivariate Cox regression analyses of the variables achieving $p < 0.20$ in univariate analyses were used to assess nivolumab impact on OS. For all analyses, $p < 0.05$ defined significance.

3. Results

3.1 Baseline characteristics

The clinical characteristics of the 59 patients are reported in Table 1. Early progressors received a median (range) of 3 infusions during 1.3 (0.5–1.8) months vs 35 (23–47) infusion during 18 (12–33) months for the long responders. Early progressors' median age was significantly older. The vast majority of the patients (90%) had an Eastern Cooperative Oncology Group Performance Status (ECOG PS) of 0/1 vs ≥2 for the remaining 10%. Early progressors

Table 1. Baseline clinical characteristics of NSCLC patients receiving nivolumab as second-or-more-line therapy.

Characteristic	General population N = 59	Early progressors N = 29	Long responders N = 30	Univariate p
Age (years), median (range)	59.5 (30.3–87.3)	65.4 (37.8–87.3)	56.8 (30.3–77.8)	0.06
Sex				0.24
Men, n (%)	44 [75]	23 [79]	21 [70]	
Women, n (%)	15 [25]	5 [17]	10 [33]	
Tobacco consumption [packs-year], mean [±SD]	37.4 ±16.9	39.1 ±14.1	36.3 ±18.6	0.26
Weight [kg], median [range]	67 [43–110]	71.5 [51–110]	62 [43–95]	0.01
Body mass index [kg/m²], median [range]	23.3 [14.9–34.3]	25.3 [17.7–34]	22.2 [14.9–34.3]	0.01
ECOG PS, n [%]				0.71
0	28 [57]	14 [48]	14 [47]	
1	25 [42]	13 [45]	12 [40]	
2	6 [10]	2 [7]	4 [13]	
Histology, n [%]				0.01
Non-squamous	47 [80]	20 [69]	27 [90]	
Squamous	12 [20]	9 [31]	3 [10]	
PD-L1 tested				0.37
No, n [%]	45 [76]	23 [79]	22 [73]	
Yes, n [%]	14 [24]	5 [17]	9 [30]	
% PD-L1 expression, median [range]	20 [0–80]	1 [0–20]	50 [1–80]	0.16
Metastatic sites				
Brain, n [%]	8 [14]	4 [14]	4 [13]	0.99
Lung, n [%]	32 [54]	14 [48]	18 [60]	0.4
Liver, n [%]	14 [24]	10 [34]	4 [13]	0.06
Adrenal gland, n [%]	7 [12]	2 [7]	5 [19/17]	0.42
Bone, n [%]	9 [15]	8 [28]	1 [3]	0.01
Pleura, n [%]	6 [10]	3 [10]	3 [10]	0.9
Prior systemic therapy, n [%]				0.57
1	49 [83]	25 [86]	24 [80]	
2	9 [15]	5 [17]	4 [13]	
≥3	1 [2]	0	1 [3]	
Platinum-based chemotherapy, n [%]	58 [98]	27 [93]	30[100]	0.47
No. of 1st-line cycles, median [range]	5 [1–23]	4.5 [2–14]	5 [1–23]	0.02
Days on 1st-line systemic therapy mean [±SD]	167 ±163	115 ±105	214 ±192	0.04
Best response to 1st-line therapy, n [%]				0.001
Partial response	22 [37]	6 [21]	16 [53]	
Stability	16 [27]	7 [24]	9 [30]	
Progression	21 [36]	15 [52]	6 [20]	
Nivolumab infusions, median n [range]	20 [2–47]	3 [2–4]	35 [23–47]	<0.0001
Months of nivolumab treatment, median [range]	11 [0.5–33]	1.3 [0.5–1.8]	18 [12–33]	<0.0001

SD, standard deviation; n, number; ECOG-PS, Eastern Cooperative Oncology Group performance status

had predominantly (69%) non-squamous NSCLCs, vs 90% for long responders ($p = 0.01$). PD-L1 testing rates were comparable for the 2 groups. Early progressors had significantly higher percentages of bone metastases ($p = 0.01$) and progressive disease ($p = 0.001$), with significantly shorter times to disease progression on previous treatment lines ($p = 0.04$).

3.2 Immunological biomarkers

Biomarker results at baseline and the 4th nivolumab infusions are given in Table 2. Baseline blood counts and NLR_{i0}, PLR_{i0}, $LIPI_{i0}$, and ALI_{i0} were comparable for the 2 groups. Almost equal numbers of long responders (11 patients, 52.2%) and early progressors (10 patients, 47.5%) had $NLR_{i0} > 5$. However, at the 4th infusion, early progressors were characterized by significantly higher ANC, and CRP and LDH concentrations, more frequent $NLR_{i4} > 5$ and higher LIPI scores. In addition, $\Delta NLR > 1$ differed significantly between the 2 groups (in univariate ($p = 0.001$) and multivariate ($p = 0.0007$) analyses). Closer examination of the 2 variables comprising the ΔNLR showed that ΔALC values differed significantly but not ΔANC.

3.3 Exploratory analyses of OS

Results of univariate and multivariate analyses of variables associated with OS are reported in Table 3. Among the parameters considered, univariate analyses selected only non-squamous histology as being associated with longer OS. $NLR_{i0} > 5$, $PLR_{i0} > 262$ and $PLR_{i4} > 262$ had no impact on OS, but $NLR_{i4} > 5$ did (hazard ratio (HR) 0.41 (95% CI 0.19–0.90), $p = 0.03$) (Fig 1). The multivariate model included histology, first-line–therapy characteristics (number of cycles, duration, best response) and ΔNLR. Three factors significantly impacted the OS prognosis: non-squamous histology, partial response to first-line systemic therapy, which directly reflects the number of first-line–therapy cycles, and $\Delta NLR > 1$. At the 4th infusion, $ANC > 6$ differed significantly but not $\Delta ANC > -0.3$. However, $\Delta ALC > 0.1$ was associated with significantly prolonged OS.

Median OS for early progressors lasted 3.1 months but was not reached for long responders (Fig 1A). For respective subgroup analyses, for $\Delta NLR > 1$, OS lasted 4.6 months vs 29.9 months (Fig 1B); for $NLR_{i0} > 5$, it lasted 7.4 months vs 29.9 months (Fig 1C); and for $NLR_{i4} > 5$, OS lasted 3.6 months vs not reached (Fig 1D).

4. Discussion

According to this novel analysis of 59 second-or-more-line nivolumab-treated NSCLC patients, NLR kinetics between the 1st and 4th infusions differed significantly between early progressors and long responders. Our results retained a significant increase of $\Delta NLR < 1$ as an independent prognostic factor, regardless of its baseline level. Moreover, they also demonstrated that a partial response to the treatment line preceding nivolumab was also associated with prolonged OS in response to the latter.

Nevertheless, we recognize some limitations of this study. First, this analysis of a moderately sized progressing population was retrospective, which carries the potential for selection bias and confounders. We attempted to contain those possible weaknesses by including only patients given nivolumab (to minimize the subtle differences among the different anti-PD-1 immunotherapies). Second, the sample size is small, which can explain some results or wide confidence intervals. Third, PD-L1 status was available for only a minority of the patients included (NSCLC PD-L1 expression was not yet sought in France when these patients were treated) but the percentages were similar into the 2 groups; no further analyses could be undertaken in this study. We also adjusted our multivariable analysis to prognostic variables, because we could not control for concomitant medications that might have influenced white blood cell counts. Fourth, unlike previously reported findings [1,2], the histology-type impact on OS mainly reflects the initial selection of more non-squamous cell NSCLCs enrolled in the long responders group. Because by definition long responders lived longer, that significantly different baseline characteristic for the 2 groups unsurprisingly affected the exploratory analysis of OS.

Table 2. NSCLC patients' biomarker values at the 2 times of interest.

Marker	General population N = 59	Early progressors N = 29	Long responders N = 30	Univariate p	Multivariate p
Just before the 1st infusion [baseline]					
Leu [Giga/L]	8.1 [2.9–30.8]	8.36 [3.1–17.67]	7.97 [2.93–30.8]	0.43	
ANC [Giga/L]	5.4 [0.7–28]	5.9 [0.69–13.94]	4.82 [1–28]	0.22	
ALC [Giga/L]	1.6 [0.3–3.3]	1.71 [0.45–2.56]	1.56 [0.29–3.34]	0.92	
APC [Giga/L]	298 [66–617]	288 [66–617]	309 [100–500]	0.57	
Albumin [g/L]	36.4 [20.8–49.9]	36 [23–49]	36 [20–44]	0.63	
CRP [mg/L]	23.5 [1–394]	27.5 [1–394]	20.5 [1–223]	0.73	
LDH [IU/L],	391 [174–679]	384 [285–597]	428 [174–679]	0.64	
NLR, n [%]					
<5	21 [36.2]	17 [59]	20 [67]	0.5	
>5	37 [63]	11 [38]	10 [33]		
PLR, n [%]					
<169	19 [32]	9 [31]	10 [33]	0.3	
169–262	31 [53]	17 [59]	14 [47]		
>262	8 [14]	2 [3]	6 [20]		
LIPI, n of patients [%]					
No. of data collected	16 [27]	9 [31]	7 [23]	0.81	
0, good	4 [25]	3 [33]	1 [14]	0.53	
1, intermediate	9 [56]	5 [56]	4 [57]		
2, poor	3 [19]	1 [11]	2 [29]		
ALI, n [%]					
<18	20 [34]	9 [31]	11 [37]	0.86	
>18	33 [56]	17 [59]	16 [53]		
Just before the 4th infusion					
Leu [Giga/L], median [range]	5 [2.7–25.1]	9.77 [4.69–25.58]	7.1 [2.7–25.1]	0.0005	
ANC [Giga/L], median [range]	5 [1.7–23.7]	7 [2.94–23.69]	3.88 [1.7–7]	<0.0001	
ALC [Giga/L], median [range]	1.7 [0.4–3.7]	1.69 [0.43–3]	1.77 [0.37–3.59]	0.29	
APC [Giga/L], n, [%] median [range]	284 [105–490]	314 [123–490]	261 [105–471]	0.19	
Albumin [g/L], median [range]	36.7 [14–48.1]	32.95 [14–48]	37.65 [27–44]	0.05	
CRP [mg/L], median [range]	22 [1–203]	46 [1–203]	13 [1–99]	0.003	
LDH [IU/L], median [range]	354 [170–758]	413 [196–758]	325 [170–441]	0.04	
NLR n, [%]					
<5	39 [66]	13 [45]	26 [87]	0.005	
>5	19 [32]	14 [48]	5 [17]		
PLR n, [%]					
<169	6 [44]	10 [34]	16 [53]	0.37	
169–262	21 [36]	10 [34]	11 [37]		
>262	11 [19]	7 [24]	4 [13]		
LIPI n, [%]					
Data collected	20 [33/34]	11 [38]	9 [30]		
0, good	7 [35]	1 [9]	6 [67]	0.01	
1, intermediate	9 [45]	6 [55]	3 [33]		
2, poor	4 [20]	4 [36]	0		
ALI n, [%]					
<18	18 [31]	5 [17]	13 [43]	0.07	
>18	34 [58]	20 [69]	14 [47]		

(Continued)

Table 2. (Continued)

Marker	General population N = 59	Early progressors N = 29	Long responders N = 30	Univariate p	Multivariate p
Evolution					
ΔNLR, median [range]	−0.18 [−17.68−+14.99]	0.65 [−13.24−+14.99]	−0.73 [−17.68−+2.91]	0.01	
ΔNLR < 1, *n* [%]	43 [73]	15 [52]	28 [93]	0.001	0.0007
ΔNLR > 1, *n* [%]	14 [24]	12 [41]	2 [7]		
ΔANC [Giga/L], mean [±SD]	−0.3 ± 5	1.3 ± 4.8	−1.7 ± 4.9	0.07	
ΔALC [Giga/L] average[±SD]	0.1 ± 0.7	−0.024 ± 0.7	0.2 ± 0.6	0.02	
ΔPLR, median [range]	2.27 [−310−+395]	16.44 [−222−+395]	−23.44 [−310−+145]	0.008	

Abbreviations: Leu, leukocytes; ANC, absolute neutrophil count; ALC, absolute lymphocyte count; APC, absolute platelet count; CRP, C-reactive protein; LDH, lactate dehydrogenase; NLR, neutrophil-to-lymphocytes ratio; PLR, platelet-to-lymphocyte ratio; LIPI, Lung Immune Prognostic Index; ALI, Advanced Lung-cancer Inflammation Index; SD, standard deviation; *n*, number.

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As emphasized in a previous study published on metastatic renal cell carcinoma patients [11], a significant increase of NLR > 25% between baseline and 6 weeks after starting immunotherapy was an independent factor predictive of shorter OS. The study published on 19 NSCLC patients [12] only reported an association between an NLR increase >30% between

Table 3. Univariate and multivariate analysis of overall survival.

Variable	Univariate analysis			Multivariate analysis		
	HR	95% CI	p value	HR	95% CI	p value
Male sex	1.83	[0.69–4.85]	0.22			
Age: >50 years	1.35	[0.51–3.58]	0.54	2.25	[0.5–10.04]	0.29
Smoking history: ≤30 pack-years	0.51	[0.16–1.56]	0.24			
ECOG-PS: 2	1.17	[0.40–3.41]	0.76			
Histology: non-squamous	0.4	[0.2–0.8]	0.01	0.19	[0.04–0.77]	0.01
PD-L1: not tested	1.77	[0.61–5.12]	0.29			
Radiotherapy: yes	1.12	[0.51–2.43]	0.78			
No. of 1st-line therapy cycles	0.88	[0.80–0.97]	0.01	0.74	[0.58–0.95]	0.01
Time on 1st-line therapy	0.996	[0.99–0.99]	0.002			
Partial response to first-line therapy	0.3	[0.12–0.75]	0.02	0.14	[0.03–0.56]	0.005
Just before the 1st infusion [baseline]						
NLR > 5	0.69	[0.32–1.94]	0.35			
PLR > 262	0.68	[0.27–1.70]	0.41			
Just before the 4th infusion						
ANC > 6 [Giga/L]	6.11	[2.6–14.18]	<0,001			
ALC > 1.5 [Giga/L]	0.99	[0.61–1.63]	0.99			
NLR > 5	0.41	[0.19–0.90]	0.03			
PLR > 262	0.87	[0.38–2]	0.74			
Evolution						
ΔNLR < 1	0.29	[0.13–0.63]	0.001	0.12	[0.03–0.46]	0.001
ΔANC > −0.3	1.468	[0.68–3.14]	0.32			
ΔALC > 0.1	0.4	[0.17–0.91]	0.03			

Abbreviations: ECOG-PS: Eastern Cooperative Oncology Group Performance Score, NLR: neutrophil-to-lymphocyte ratio, PLR: platelet-to-lymphocyte ratio, ANC: absolute neutrophil count, ALC: absolute lymphocyte count, ΔNLR: $NLR_{i4} - NLR_{i0}$, ΔANC: $ANC_{i4} - ANC_{i0}$, ΔALC: $ALC_{i4} - ALC_{i0}$

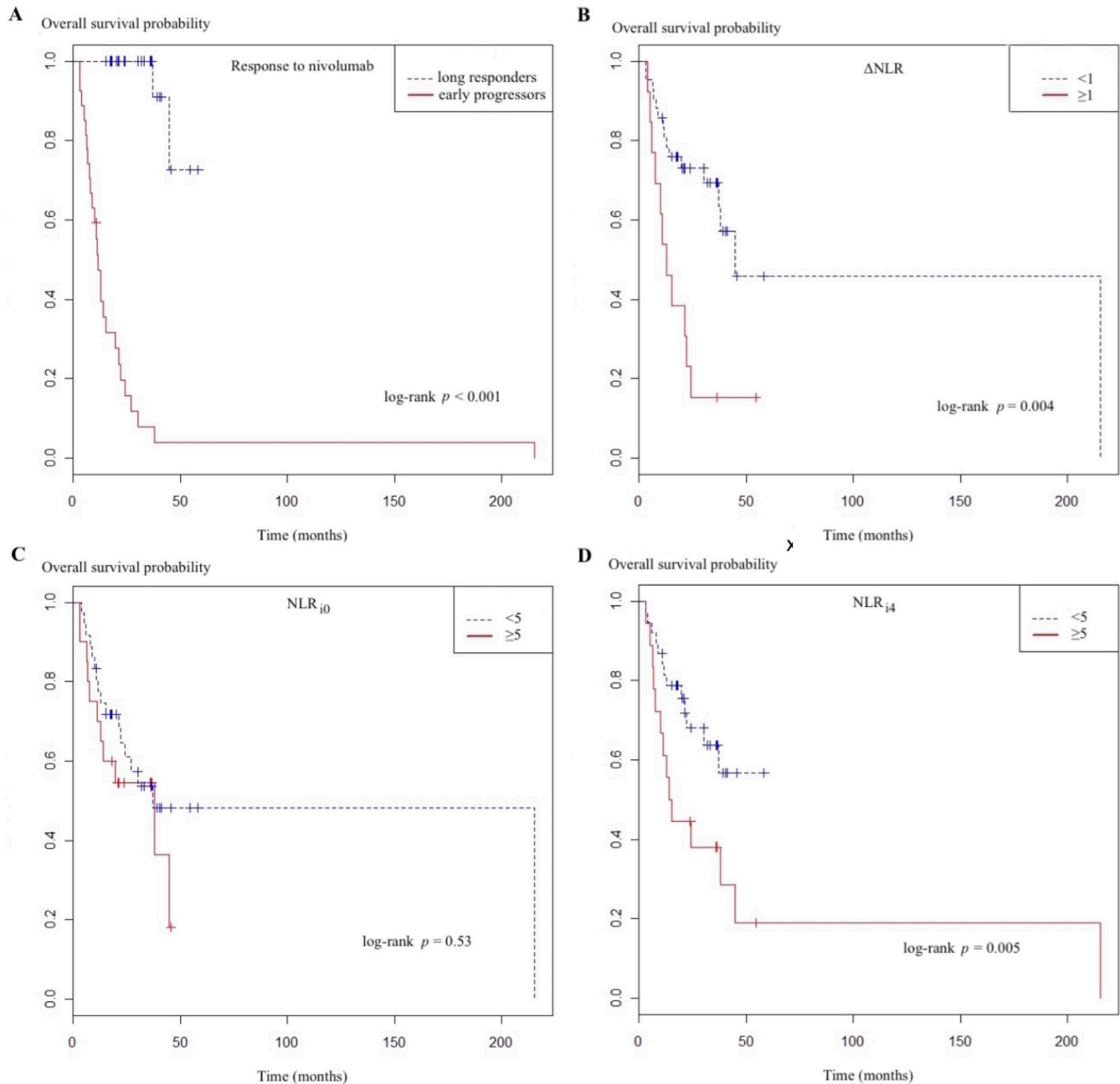https://doi.org/10.1371/journal.pone.0219060.t003

Fig 1. Overall survival probability, according to the response to nivolumab (A), ΔNLR (B), NLR_{i0} (C), and NLR_{i4} (D), (A) response to Nivolumab, (B) ΔNLR (NLR change between the 1st and 4th nivolumab infusions), (C) NLR_{i0} (neutrophil-to-lymphocytes ratio; i0, just before the 1st nivolumab infusion), ((D) NLR_{i4} (neutrophil-to-lymphocytes ratio i4, just before 4th nivolumab infusion.

the 1st and 2nd ($p = 0.014$), and 2nd and 3rd immunotherapy infusions ($p < 0.001$) and shorter times to treatment failures; no association was found between NLR evolution and OS or PFS. Other than the smaller number and the older age of the patients that they included, the majority had recurrent or stage-III NSCLCs and received immunotherapy later during their care (3rd-or-more treatment line).

The first studies to demonstrate a clear association between NLR and OS focused on NLR at baseline [7,8] and 6 weeks [9]. The former two studies retained a cut-off threshold of $\geq$5 but they respectively divided them into tertiles or quartiles, yielding other possible cut-off options. Those results were counterbalanced by the study highlighting the importance of NLR at 6 weeks [9], in which no associations between OS and baseline NLR were identified. Our similar population in terms of ECOG PS and previous treatment lines, our median OS of 3.6 months for $NLR_{i4} > 5$ was close to the 2.1 months previously reported [9].

Our selected population had many characteristics in common with the patients enrolled in phase-III trials that led to nivolumab approval for NSCLC treatment [1,2]: median age of 59.5 years compared to 62 years, a small minority of patients with ECOG PS > 2 (10% vs none) and about the same percentage of patients with $\geq$2 prior treatment lines (17% vs 12%).

Furthermore, the response to first-line chemotherapy before nivolumab had also been described previously for melanoma [15] and NSCLC [7,16–18]. Those responses could be caused by antigens released after tumor-cell death that would stimulate the immune T-cell response and enhance the immunotherapy mechanism of action [19–21]. We included all the previously identified clinical parameters in our multivariate analyses and they remained significant.

Previous studies on NSCLC patients treated with surgery, chemoradiotherapy or chemotherapy alone showed that a high NLR is an independent factor predictive of shorter OS and poorer PFS [22–25]. This phenomenon could be explained by an antigen-driven immune response with one or more suppressive factors [26] within the tumor microenvironment, which would lead to an immune dysfunction. The immunosuppressive tumor cells in the microenvironment, such as myeloid-derived suppressor cells or tumor-associated neutrophils [6], might be involved. Those cell types showed characteristics close to those of circulating neutrophils [27], and their influence on lymphocytes [28,29] and tumor growth [30] has been proven.

Precedent studies have underlined the positive impact of higher body mass index (BMI) >25kg/m2 on OS under chemotherapy (Carboplatin Taxol) [31] for NSCLC. A study [32] on melanoma proved similar results under immunotherapy. BMI was analyzed at a single point, without studying potential previous weight loss. In our study, BMI was collected at the diagnosis, before the first line of treatment. Since most early progressors did not respond to their first line of treatment, their performans status and weight mostly decreased, but we could not obtain a sufficient data and sample sizes to properly assess reliable statistics at the time of immunotherapy start. These results only reflects the baseline characteristics at the tumor diagnosis.

Concerning the particular NSCLC immune landscape, a recent publication [33] found a predominance of neutrophils. However, we had comparable numbers of patients in each group with $NLR_{i0} > 5$. Moreover, we found a significantly higher ANC_{i4}, but the $\Delta ALC > -0.3$ had no impact on OS. The evolution of $\Delta ALC > 0.1$ differed statistically between our 2 study groups. Overall, a high pretreatment ANC, previously described by Bagley et al [8], and at the 4th infusion in our study, was associated with shorter OS, without evolving notably over time. As stated above, $\Delta ALC > 0.1$ after starting nivolumab was associated with longer OS. That finding led us to wonder whether nivolumab would still able to overcome an immune response once dominated by neutrophils and how to determine the optimal threshold of where nivolumab could be effective: can baseline NLR adequately define the immune response and predict the response to nivolumab, or would ΔNLR better characterize each patient and be more accurately establish the cut-off?

Future prospective studies on larger populations comparing these two approaches are warranted. Because nivolumab does not seem to be effective in patients with high inflammatory

(neutrophil) status, further prospective studies concerning this specific population should also be conducted to clarify the treatment algorithm. For example, comparing nivolumab versus classical chemotherapy, like paclitaxel, for progressing patients with significantly elevated NLR between the 1st and 4th nivolumab infusions could informative.

5. Conclusion

The results of this original study comparing only early progressor to long responder NSCLC patients demonstrated the importance of ΔNLR as an independent factor prognostic of OS. Early progressors were characterized by ΔNLR > 1 and progression as the best response to prior treatment line. NLR evolution was also shown to have an independent influence on OS. An increasing body of evidence seems to underline the central role of neutrophils in tumor aggressiveness and the inability of nivolumab to stop and overturn neutrophils' pro-tumor action. Further studies on larger patient populations are needed to clarify the potential use of inflammatory biomarker evolution before and under immunotherapy as predictive and prognostic indicators of outcome.

Supporting information

S1 File. Responders data set [French version].
(XLSX)

S2 File. Hyperprogressors data set [French version].
(XLSX)

Acknowledgments

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References

1. Brahmer J, Reckamp KL, Baas P, Crinò L, Eberhardt WEE, Poddubskaya E, et al. Nivolumab versus Docetaxel in Advanced Squamous-Cell Non–Small-Cell Lung Cancer. N Engl J Med. 2015 Jul 9; 373 [2]:123–35. https://doi.org/10.1056/NEJMoa1504627 PMID: 26028407

2. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. N Engl J Med. 2015 Oct 22; 373[17]:1627–39. https://doi.org/10.1056/NEJMoa1507643 PMID: 26412456

3. Messerschmidt JL, Prendergast GC, Messerschmidt GL. How Cancers Escape Immune Destruction and Mechanisms of Action for the New Significantly Active Immune Therapies: Helping Nonimmunologists Decipher Recent Advances. The Oncologist. 2016 Feb 1; 21[2]:233–43. https://doi.org/10.1634/theoncologist.2015-0282 PMID: 26834161

4. Ohaegbulam KC, Assal A, Lazar-Molnar E, Yao Y, Zang X. Human cancer immunotherapy with anti-bodies to the PD-1 and PD-L1 pathway. Trends Mol Med. 2015 Jan; 21[1]:24–33. https://doi.org/10.1016/j.molmed.2014.10.009 PMID: 25440090

5. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJM, Robert L, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature. 2014 Nov 27; 515[7528]:568–71. https://doi.org/10.1038/nature13954 PMID: 25428505

6. Shaul ME, Fridlender ZG. Cancer related circulating and tumor-associated neutrophils—subtypes, sources and function. FEBS J. 2018 May 31;

7. Diem S, Schmid S, Krapf M, Flatz L, Born D, Jochum W, et al. Neutrophil-to-Lymphocyte ratio [NLR] and Platelet-to-Lymphocyte ratio [PLR] as prognostic markers in patients with non-small cell lung cancer [NSCLC] treated with nivolumab. Lung Cancer Amst Neth. 2017 Sep; 111:176–81.

8. Bagley SJ, Kothari S, Aggarwal C, Bauml JM, Alley EW, Evans TL, et al. Pretreatment neutrophil-to-lymphocyte ratio as a marker of outcomes in nivolumab-treated patients with advanced non-small-cell lung cancer. Lung Cancer Amst Neth. 2017; 106:1–7.

9. Suh KJ, Kim SH, Kim YJ, Kim M, Keam B, Kim TM, et al. Post-treatment neutrophil-to-lymphocyte ratio at week 6 is prognostic in patients with advanced non-small cell lung cancers treated with anti-PD-1 antibody. Cancer Immunol Immunother CII. 2017 Dec 4;

10. Parikh K, Kumar A, Ahmed J, Anwar A, Puccio C, Chun H, et al. Peripheral monocytes and neutrophils predict response to immune checkpoint inhibitors in patients with metastatic non-small cell lung cancer. Cancer Immunol Immunother CII. 2018 Jul 2;

11. Lalani A-KA, Xie W, Martini DJ, Steinharter JA, Norton CK, Krajewski KM, et al. Change in Neutrophil-to-lymphocyte ratio [NLR] in response to immune checkpoint blockade for metastatic renal cell carcinoma. J Immunother Cancer. 2018 Jan 22; 6[1]:5. https://doi.org/10.1186/s40425-018-0315-0 PMID: 29353553

12. Kiriu T, Yamamoto M, Nagano T, Hazama D, Sekiya R, Katsurada M, et al. The time-series behavior of neutrophil-to-lymphocyte ratio is useful as a predictive marker in non-small cell lung cancer. Coleman WB, editor. PLOS ONE. 2018 Feb 15; 13[2]:e0193018. https://doi.org/10.1371/journal.pone.0193018 PMID: 29447258

13. Shiroyama T, Suzuki H, Tamiya M, Tamiya A, Tanaka A, Okamoto N, et al. Pretreatment advanced lung cancer inflammation index [ALI] for predicting early progression in nivolumab-treated patients with advanced non-small cell lung cancer. Cancer Med. 2018 Jan; 7[1]:13–20. https://doi.org/10.1002/cam4.1234 PMID: 29150906

14. Mezquita L, Auclin E, Ferrara R, Charrier M, Remon J, Planchard D, et al. Association of the Lung Immune Prognostic Index With Immune Checkpoint Inhibitor Outcomes in Patients With Advanced Non-Small Cell Lung Cancer. JAMA Oncol. 2018 Mar 1; 4[3]:351–7. https://doi.org/10.1001/jamaoncol.2017.4771 PMID: 29327044

15. Heidelberger V, Goldwasser F, Kramkimel N, Jouinot A, Franck N, Arrondeau J, et al. Clinical parameters associated with anti-programmed death-1 [PD-1] inhibitors-induced tumor response in melanoma patients. Invest New Drugs. 2017; 35[6]:842–7. https://doi.org/10.1007/s10637-017-0476-6 PMID: 28569347

16. Facchinetti F, Veneziani M, Buti S, Gelsomino F, Squadrilli A, Bordi P, et al. Clinical and hematologic parameters address the outcomes of non-small-cell lung cancer patients treated with nivolumab. Immunotherapy. 2018 Jun; 10[8]:681–94. https://doi.org/10.2217/imt-2017-0175 PMID: 29882691

17. Kobayashi H, Omori S, Nakashima K, Wakuda K, Ono A, Kenmotsu H, et al. Response to the treatment immediately before nivolumab monotherapy may predict clinical response to nivolumab in patients with non-small cell lung cancer. Int J Clin Oncol. 2017 Aug; 22[4]:690–7. https://doi.org/10.1007/s10147-017-1118-x PMID: 28382561

18. Garde-Noguera J, Martin-Martorell P, De Julián M, Perez-Altozano J, Salvador-Coloma C, García-Sanchez J, et al. Predictive and prognostic clinical and pathological factors of nivolumab efficacy in non-small-cell lung cancer patients. Clin Transl Oncol Off Publ Fed Span Oncol Soc Natl Cancer Inst Mex. 2018 Aug; 20[8]:1072–9.

19. Emens LA, Middleton G. The interplay of immunotherapy and chemotherapy: harnessing potential synergies. Cancer Immunol Res. 2015 May; 3[5]:436–43. https://doi.org/10.1158/2326-6066.CIR-15-0064 PMID: 25941355

20. Obeid M, Panaretakis T, Tesniere A, Joza N, Tufi R, Apetoh L, et al. Leveraging the immune system during chemotherapy: moving calreticulin to the cell surface converts apoptotic death from "silent" to immunogenic. Cancer Res. 2007 Sep 1; 67[17]:7941–4. https://doi.org/10.1158/0008-5472.CAN-07-1622 PMID: 17804698

21. Zitvogel L, Kepp O, Kroemer G. Immune parameters affecting the efficacy of chemotherapeutic regimens. Nat Rev Clin Oncol. 2011 Mar; 8[3]:151–60. https://doi.org/10.1038/nrclinonc.2010.223 PMID: 21364688

22. Derman BA, Macklis JN, Azeem MS, Sayidine S, Basu S, Batus M, et al. Relationships between longitudinal neutrophil to lymphocyte ratios, body weight changes, and overall survival in patients with non-small cell lung cancer. BMC Cancer. 2017 16; 17[1]:141. https://doi.org/10.1186/s12885-017-3122-y PMID: 28209123

23. Peng B, Wang Y-H, Liu Y-M, Ma L-X. Prognostic significance of the neutrophil to lymphocyte ratio in patients with non-small cell lung cancer: a systemic review and meta-analysis.:9.

24. Scilla KA, Bentzen SM, Lam VK, Mohindra P, Nichols EM, Vyfhuis MA, et al. Neutrophil-Lymphocyte Ratio Is a Prognostic Marker in Patients with Locally Advanced [Stage IIIA and IIIB] Non-Small Cell Lung Cancer Treated with Combined Modality Therapy. The Oncologist. 2017 Jun; 22[6]:737–42. https://doi.org/10.1634/theoncologist.2016-0443 PMID: 28533476

25. Zhao Q-T, Yang Y, Xu S, Zhang X-P, Wang H-E, Zhang H, et al. Prognostic role of neutrophil to lymphocyte ratio in lung cancers: a meta-analysis including 7,054 patients. OncoTargets Ther. 2015; 8:2731–8.

26. de Visser KE, Eichten A, Coussens LM. Paradoxical roles of the immune system during cancer development. Nat Rev Cancer. 2006 Jan; 6[1]:24–37. https://doi.org/10.1038/nrc1782 PMID: 16397525

27. Brandau S, Moses K, Lang S. The kinship of neutrophils and granulocytic myeloid-derived suppressor cells in cancer: Cousins, siblings or twins? Semin Cancer Biol. 2013 Jun 1; 23[3]:171–82. https://doi.org/10.1016/j.semcancer.2013.02.007 PMID: 23459190

28. Lu T, Ramakrishnan R, Altiok S, Youn J-I, Cheng P, Celis E, et al. Tumor-infiltrating myeloid cells induce tumor cell resistance to cytotoxic T cells in mice. J Clin Invest. 2011 Oct; 121[10]:4015–29. https://doi.org/10.1172/JCI45862 PMID: 21911941

29. Yan HH, Pickup M, Pang Y, Gorska AE, Li Z, Chytil A, et al. Gr-1+CD11b+ myeloid cells tip the balance of immune protection to tumor promotion in the premetastatic lung. Cancer Res. 2010 Aug 1; 70 [15]:6139–49. https://doi.org/10.1158/0008-5472.CAN-10-0706 PMID: 20631080

30. Whiteside TL. The tumor microenvironment and its role in promoting tumor growth. Oncogene. 2008 Oct 6; 27[45]:5904–12. https://doi.org/10.1038/onc.2008.271 PMID: 18836471

31. McQuade JL, Daniel CR, Hess KR, Mak C, Wang DY, Rai RR, et al. Association of body-mass index and outcomes in patients with metastatic melanoma treated with targeted therapy, immunotherapy, or chemotherapy: a retrospective, multicohort analysis. The Lancet Oncology. 2018; 19: 310–322. https://doi.org/10.1016/S1470-2045(18)30078-0 PMID: 29449192

32. Greenlee H, Unger JM, LeBlanc M, Ramsey S, Hershman DL. Association between Body Mass Index and Cancer Survival in a Pooled Analysis of 22 Clinical Trials. Cancer Epidemiol Biomarkers Prev. 2017; 26: 21–29. https://doi.org/10.1158/1055-9965.EPI-15-1336 PMID: 27986655

33. Kargl J, Busch SE, Yang GHY, Kim K-H, Hanke ML, Metz HE, et al. Neutrophils dominate the immune cell composition in non-small cell lung cancer. Nat Commun. 2017 Feb 1; 8:14381. https://doi.org/10.1038/ncomms14381 PMID: 28146145