Long title:

Neutrophil-lymphocyte ratio (NLR) kinetics in patients with advanced solid tumours on phase I trials of PD-1/PD-L1 inhibitors

Short title:

NLR kinetics in phase I patients on PD-1/PD-L1 inhibitors

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ABSTRACT:

Background:

Although the neutrophil-lymphocyte ratio (NLR) is prognostic in many oncological settings, its significance in the immunotherapy era is unknown. Mechanistically, PD-1/PD-L1 inhibitors may alter NLR. We sought to characterise NLR kinetics in patients with advanced solid tumours treated with PD-1/PD-L1 inhibitors.

Methods:

Electronic records of patients treated with PD-1/PD-L1 inhibitors on phase I trials across three sites were reviewed. A high NLR (hNLR) was predefined as >5. Univariate logistic regression models were used for toxicity, response analyses and Cox models for overall survival (OS) and progression-free survival analyses. Landmark analyses were performed (cycle two, three). Longitudinal analysis of NLR was performed utilising a mixed effect regression model.

Results:

The median OS for patients with hNLR was 8.5 months and 19.4 for patients with low NLR, (HR= 1.85, 95%CI 1.15-2.96, p=0.01). On landmark analysis, hNLR was significantly associated with inferior OS at all timepoints with a similar magnitude of effect over time ($p<0.05$). On multivariate analysis, NLR was associated with OS (HR 1.06, 95% CI 1.01-1.11, p=0.01). NLR did not correlate with increased immune toxicity. Longitudinally, NLR correlated with response: NLR decreased by 0.09 (95% CI: -0.15 to -0.02; p=0.01) per month in responders compared to non-responders.

Conclusions:

hNLR at baseline and during treatment is adversely prognostic in patients with advanced malignancies receiving PD-1/PD-L1 blockade. Importantly, NLR reduced over time in responders to immunotherapy. Taken together, these data suggest that baseline and longitudinal NLR may have utility as a unique biomarker to aid clinical decision-making in patients receiving immunotherapy.

Background:

The advent of new cancer immunotherapeutics has led to renewed optimism in clinically meaningful progress to improve cancer outcomes for patients. In particular, since a survival benefit was observed with ipilimumab, an immune checkpoint inhibitor (ICI), in patients with advanced melanoma (1), there has been an explosion in novel cancer immunotherapeutics. The most successful molecules to date have been inhibitors of the programmed-death 1 (PD-1) - programmed death-ligand 1 (PD-L1) checkpoint, which have shown efficacy in multiple tumour types (2-5). The success of these agents has launched a plethora of clinical trials in additional tumour types (6), in novel combinations to combat resistance (7), and increasingly in earlier treatment settings (8). Clinically, as these therapeutics enter standard practice, there will be a challenge, as the response and toxicity profiles of these agents differ substantially from those of traditional cytotoxics and targeted therapies (9, 10).

The neutrophil-lymphocyte ratio (NLR) is a widely available blood-based clinical biomarker. It is validated in numerous oncological settings (11), including the phase I setting (12), as both a prognostic marker (13) and a predictor of benefit for systemic therapy (14, 15). Its clinical relevance is thought to stem from the fact that an elevated peripheral neutrophil count (the numerator for the NLR), is a marker of chronic inflammation which often leads to impaired immunity (16), whilst conversely the peripheral lymphocyte count (the denominator), is a hallmark of a healthy cytotoxic T-cell (CTL) response. Increasingly, the interaction between the various populations of immune cells has been recognised as critical in forming the immune microenvironment, which forms the milieu through which the anti-cancer immune response occurs (17). Moreover, as novel immunotherapeutics are licensed and enter clinical practice, new insights on the mechanisms underlying these interactions is emerging. For instance, it is increasingly recognised that neutrophils directly suppress CTL activity via the release of cytokines (16), and that neutrophils stimulated by

interferon-gamma are more likely to impair CTLs than other neutrophils. Holistically, a lower NLR, which has consistently been shown to correlate with improved cancer outcomes (18), is thought to delineate a healthy host immune anti-tumour response.

Nevertheless, there are several limitations with the NLR. Historically, most studies correlating the NLR with survival outcomes in cancer have used the baseline NLR prior to treatment as a static measure (18). However, the NLR appears to be dynamic and there is sparse data on the kinetics of the NLR response. Some of the largest studies to date have been performed in the urology literature, which suggest a decreasing NLR correlates with improved outcomes in patients receiving anti-angiogenic therapies (19). Other studies have shown that a decreasing NLR during chemoradiotherapy for glioblastoma is associated with improved survival (20), and that increases in NLR in patients undergoing palliative care are associated with inferior survival (21). However, there is no published data regarding dynamic NLR kinetics with ICIs. Dynamic changes in NLR pose a particular challenge with immunotherapy, as there is the possibility that a change in NLR may reflect a mechanistic response to therapy. To date, initial studies have demonstrated that baseline NLR has prognostic value in melanoma patients treated with ipilimumab (22), and that baseline NLR has prognostic value in lung cancer patients treated with nivolumab (23).

Moreover, given the emergence of the concept of pseudo-progression (10), there is an important clinical need to identify non-imaging based biomarkers that suggest response or progression on therapy. Additionally, given the unique profile of immune toxicity (9), blood-based early biomarkers of emergent immune toxicity may also find a place in the clinical armamentarium. The primary aim of this study was therefore, to evaluate the relationship of baseline NLR with response, survival and toxicity in patients with advanced solid tumours in patients treated in the phase I setting with antagonists of the PD-1/PD-L1 checkpoint. The secondary aim was to evaluate the longitudinal

kinetics of the NLR in these patients. The exploratory aim was to evaluate whether dynamic changes in the NLR were being driven by a change in the absolute lymphocyte count or the absolute neutrophil count.

Methods:

Study design:

We conducted a retrospective multi-centre cohort study of consecutive patients with advanced solid tumours treated with PD-1/PD-L1 inhibitors in phase I clinical trials across three sites in the United Kingdom, Spain and Australia. At least one dose of experimental agent needed to be received to be eligible for inclusion on this study. Data collected included patient demographics and clinical data, haematology and biochemistry at baseline and prior to each cycle of therapy, toxicity data, concomitant medications, response data, date of last follow-up and date of death. NLR at each cycle was calculated as the total neutrophil count divided by the total lymphocyte count. A high NLR (hNLR) was defined as an NLR ≥ 5 consistent with the published literature (18, 23) and a low NLR (lNLR) as an NLR <5. Adverse events (AEs) were graded according to the Common Terminology Criteria for Adverse Events (CTCAE v4.03) and were separated into immune-related and other toxicity. Corticosteroid use, start date and indication were recorded. Overall survival (OS) was calculated from date of first treatment to date of death and was censored at date of last follow-up. Progression-free survival (PFS) was calculated from first treatment to date of first progression by RECIST 1.1 and was censored at date of last follow-up. Clinical benefit (CB) was defined as best response of complete response (CR)/partial response (PR) or stable disease (SD) by RECIST 1.1 (24), and was utilised over response rate as there is some evidence that SD is evidence of anti-tumour activity with immunotherapeutics (10). Ethics approval for the study was obtained by each participating institution.

Statistical analysis:

Fisher's exact test and Wilcoxon rank-sum test were used respectively to describe the association of continuous and categorical variables with baseline NLR status. Univariate logistic regression analyses were used to describe the association between NLR and response, toxicity and predefined established prognostic variables. Kaplan-Meier plots present OS and PFS by baseline NLR status. Univariate and multivariate Cox models were used in OS and PFS analyses. Variables where $p<0.1$ in univariate analyses were selected for inclusion in multivariate analysis; baseline lymphocyte, ANC and NLR status were however excluded due to the relationship with baseline NLR. Baseline albumin and LDH were excluded from multivariate analyses since these factors form part of the prognostically validated RMH score. The proportional hazards assumption was assessed using Schoenfeld residuals. A landmark analysis was performed for NLR at cycle two and three for both response and survival analyses. Survival and logistic analyses were conducted using Stata v13.1.

For the longitudinal analysis, a mixed effect regression model with per-patient random intercept and slope was applied to longitudinal NLR. To enable better model fit NLR was transformed to be normally-distributed using a zero-skewness log transformation (transformed NLR=ln(NLR-0.29)). Due to the possible impact of corticosteroids on absolute neutrophil count, additional analyses were performed with data censored at steroid commencement date. Additionally, a longitudinal analysis of absolute lymphocyte count was performed as an exploratory analysis, using *R* v3.3.2.

***Results**:*

Between May 2014 and May 2017, 165 patients were treated with PD-1 and PD-L1 inhibitors. Baseline characteristics are displayed in Table 1. Baseline characteristics were well balanced by

baseline NLR status although patients with higher baseline NLR were more likely to have received previous radiotherapy treatment (p=0.03). Patients with higher baseline NLR were observed to have lower baseline haemoglobin (p<0.001) and lower baseline albumin (p<0.001).

Overall, 79/165 (48%) achieved a CB. Results from univariate logistic regression models of CB are shown in Table 2. On univariate logistic regression model for CB, there was no evidence that gender, age, PD-1 versus PD-L1 inhibitors, previous surgery or previous radiotherapy influenced response. A higher baseline NLR was associated with lower odds of CB, driven largely by changes in the baseline lymphocyte count. On multivariate analyses, after accounting for differences in baseline haemoglobin, there was no evidence of a difference in CB by baseline NLR. However, baseline haemoglobin and NLR were highly correlated at baseline (Table 1).

On landmark (Baseline, Cycle 2 and Cycle 3) logistic regression analyses for CB and univariate Cox models for OS, hNLR at was associated with a decreased odds ratio for clinical benefit at all timepoints and an increased risk of mortality on survival analysis, with a similar magnitude observed over time (Table 3).

Median overall survival was 12.8 months (95% CI: 9.9-19.4). Median survival for patients with low NLR (lNLR) was 19.4 months (95% CI 11.2-21.0) compared to 8.5 (95% CI 4.4-13.0) months for hNLR patients. Univariate Cox models were performed for OS and PFS (Table 4 and Table 5) and the corresponding Kaplan-Meier plots are shown in Figure 1. Baseline hNLR was associated with inferior OS (HR=1.85, 95% CI 1.15-2.96, p=0.01) but not with PFS (HR=1.14; 95% CI: 0.79-1.65; p=0.48). On multivariate analyses, baseline NLR and RMH score were associated with OS (Table 4); each unit increase in NLR lead to a 6% increase (HR=1.06; 95% CI=1.02-1.11; p=0.008) in the incidence of mortality.

We also evaluated outcomes related to toxicity. Previous surgery, baseline lymphocyte count and RMH score were associated with immune toxicity (see Table 6) but were not associated with grade 3/4 immune toxicity. Only baseline ANC was associated with grade 3/4 immune toxicity.

We subsequently analysed NLR over time. A mixed-effects regression analysis with per patient random intercept was used to analyses changes in NLR during follow-up. Patients were censored at the start date of steroid use to exclude the confounding effect of steroids on total neutrophil count. A spaghetti plot of transformed NLR is shown in Figure 2. Overall, patients with CB had a lower log transformed NLR (p=0.03) at study entry (Coef=-0.39; 95% CI=-0.66 to -0.12; p=0.005 and Coef=-0.39; 95% CI=-0.61 to -0.17; p<0.001 respectively) compared to patients with progressive disease (PD). There was no evidence for an overall change in transformed NLR during follow-up (Coef=-0.01; 95% CI=-0.08 to 0.05; p=0.71).

On exploratory analysis, however, there was evidence that the change in transformed NLR correlated with response (p=0.03). Patients with a RECIST response of CR/PR, had a -0.07 (95% CI=-0.16 to 0.02; p=0.12) change in transformed NLR per month compared to patients with PD, while patients with SD did not (Coef=0.03; 95% CI=-0.05 to 0.11; p=0.49). Compared to the combined group of patients with SD and PD, patients with CR/PR had a -0.09 (95% CI: -0.15 to -0.02; p=0.01) change in transformed NLR per month. There was no evidence of a quadratic change in transformed NLR over time. We applied the model to predict NLR by response category (see Figure 3), which suggests that responders typically have a reduction in NLR over time compared to non-responders and patients with stable disease. Finally, we attempted to delineate whether the dynamic changes in NLR over time were predominantly due to increased lymphocytes versus decreased neutrophils in responders. Overall, there was no statistically significant evidence of changes in lymphocytes or

neutrophils over time, suggesting that the observed change in NLR was due to a combination of changes in both populations of immune cells.

Discussion

The NLR is a widely validated clinical biomarker of prognosis applicable to several clinical settings. Its role in the immunotherapy era is undefined, although studies have shown that for melanoma patients treated with the CTLA-4 inhibitor, ipilimumab, the NLR retains its prognostic significance (22). This study suggests that baseline NLR is correlated with clinical benefit and that it is significantly prognostic for overall survival in a population of patients with advanced solid tumours participating in phase I trials of PD-1/PD-L1 inhibitors. Although a significant effect was not observed in PFS analyses, given the relative clinical significance of OS and the difficulties in assessing PFS in patients treated with immunotherapeutics given novel patterns of response (10), the significance of the OS data cannot be understated.

Moreover, given the evident potential impact of immunotherapy on the dynamic NLR, this is the first study, to our knowledge, analysing the dynamic kinetics of the NLR. Firstly, it was reassuring to note that the NLR retained its broadly prognostic significance over time in the landmark analysis, with a consistent effect size temporally. This continued effect was subsequently explained by the mixed-effects regression analysis which showed that responding patients had a consistent decrease in the NLR over time, whereas patients with stable disease or progression did not. This analysis was not coloured by steroid utilisation artificially increasing the neutrophil count in patients with steroid-responsive disease-related symptoms or toxicity as data was censored at steroid commencement date. This is the first study to report this finding in patients receiving PD-1/PD-L1 inhibitors. Importantly, as has been recognised elsewhere (25), the escalating costs of immunotherapy to the

overall burden of healthcare costs is unsustainable, yet in this very context, clinicians are struggling to cease potentially ineffectual therapies due to the perceived risk of missing pseudo-progression (26). With this additional data in mind, it may be useful for clinicians to recognise that a combination of a disease progression by traditional radiographic response criteria and a rising or high NLR represents a clinical scenario with a low likelihood of benefit from continued therapy. Moreover, the negative finding that clinical variables and the NLR are not predictive of treatment emergent toxicity is noteworthy.

The results of this study also pose further scientific questions. Significantly, the mechanism by which dynamic changes in NLR were correlated with improved prognosis was not explained purely by an increase in the absolute lymphocyte count alone, as might be inferred given the mechanism of action of PD-1 inhibitors. Nor was it explained by a decrease in the neutrophil count, but rather, by a change in both subsets. These findings suggest that the change in NLR observed in responders may reflect broader changes in the tumour microenvironment, the exact nature of which remains to be further elucidated.

There are several limitations to the study. Firstly, this study was limited to patients with advanced solid tumours participating in phase I clinical trials. Although the results may not be generalisable to a general oncology population, there was a wide range of solid tumours represented. Moreover, the fact that this was a multi-institutional, multi-national collaboration of phase I trial centres, ensured high quality and complete data was maintained prospectively, with minimal missing data skewing the analysis. Moreover, given the retrospective nature of the study, if the dynamic NLR is to shift clinical practice to be utilised as an additional clinical biomarker in decision-making, this data needs to be validated, ideally from a prospectively collected independent dataset.

As PD-1 inhibitors increasingly enter routine clinical practice, the importance of readily available clinical biomarkers to assist clinical decision making is paramount. This study shows that the NLR is significantly prognostic on overall survival outcomes on patients with advanced solid tumours participating in phase I trials of PD-1/PD-L1 inhibitors. Furthermore, it is the first study to suggest that responding patients have a decline in their longitudinal NLR over time, whereas patients who fail to respond do not, a fact which may assist clinical decision making.

Conflict of interest statement

We have no conflicts of interest to disclose with regards to this research.

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Table 1: Baseline characteristics

Variable	Baseline NLR≤5 N	Baseline NLR≤5 %	Baseline NLR>5 N	Baseline NLR>5 %	p-value
Total	110		55		
Gender					
Female	46	42	28	51	0.32
Male	64	58	27	49	
Treatment type					
PD1	99	90	52	95	0.39
PD-L1	11	10	3	5	
Tumour Type					
Gastrointestinal	14	13	10	18	
Lung	38	35	13	24	
Mesothelioma	15	14	9	16	
Gynaecological	15	14	14	25	0.25
Central nervous system	2	2	0	0	
Genitourinary	7	6	4	7	
Other	19	17	5	9	
Previous Surgery					
No	61	55	32	58	0.87
Yes	49	45	23	42	
Previous Radiotherapy					
No	73	66	26	47	**0.03**
Yes	37	34	29	53	
	Median	**Q1-Q3**	**Median**	**Q1-Q3**	
Age (years)	62	57-70	61	49-71	0.30
Baseline Hb (g/L)	121	113-132	110	102-121	**<0.001**
Albumin (g/L)	38	34-41	34	30-38	**<0.001**
Log_{10} LDH	2.6	2.3-2.8	2.5	2.2-2.7	0.09

Table 2: Univariate and multivariate regression analysis for clinical benefit (CB)

Variables	Uni OR	95% CI	p-value	Multi OR	95% CI	p-value
Male vs. female	1.04	0.56-1.93	0.89	-	-	-
PD-L1 inhibitor vs. PD-1 inhibitor	1.50	0.50-4.54	0.47	-	-	-
Previous surgery (yes vs. no)	0.86	0.47-1.60	0.64	-	-	-
Previous radiotherapy (yes vs. no)	0.85	0.46-1.59	0.61	-	-	-
Age (by decade)	1.04	0.83-1.30	0.75	-	-	-
Baseline NLR	0.91	0.83-0.99	**0.04**	0.93	0.85-1.02	0.11
Baseline ANC	0.96	0.86-1.07	0.50	-	-	-
Baseline Lymph	1.67	0.93-3.00	0.08	-	-	-
Baseline Hb (g/L)	1.03	1.01-1.05	**0.008**	1.02	1.00-1.04	**0.04**
Albumin (g/L)	1.09	1.02-1.15	**0.008**	-	-	-
Log_{10} LDH (U/L)	0.51	0.19-1.33	0.17	-	-	-
RMH Score	0.71	0.50-1.01	0.06	0.74	0.52-1.07	0.11

Table 3: Landmark analysis for NLR for CB and OS.

NLR timepoint	N	Landmark analysis for CB			Landmark analysis for OS		
		OR	95% CI	p-value	HR	95% CI	p-value
Baseline	165	0.91	0.83-0.99	**0.04**	1.07	1.03-1.11	**0.001**
Cycle 2	150	0.91	0.82-1.00	**0.05**	1.06	1.01-1.11	**0.01**
Cycle 3	129	0.88	0.79-0.98	**0.02**	1.06	1.01-1.11	**0.02**

Table 4: Univariate and multivariate Cox models **for OS**

Variables	Uni HR for OS	95% CI	p-value	Multi HR for OS	95% CI	p-value
Gender						
Male vs. female	0.95	0.59-1.54	0.85	-	-	-
Treatment						
PD-L1 inhibitor vs. PD-1 inhibitor	1.44	0.62-3.35	0.40	-	-	-
Previous Surgery (yes vs. no)						
Yes	0.99	0.61-1.58	0.95	-	-	-
Previous Radiotherapy (yes vs. no)						
	0.95	0.58-1.53	0.83	-	-	-
Baseline NLR>5						
hNLR vs lNLR	1.85	1.15-2.96	**0.01**	-	-	-
Age (10 Years)	1.05	0.89-1.25	0.54	-	-	-
Baseline NLR	1.07	1.03-1.11	**0.001**	1.06	1.02-1.11	**0.008**
Baseline ANC	1.05	0.99-1.11	0.13	-	-	-
Baseline Lymph	0.64	0.40-1.04	0.07	-	-	-
Baseline Hb	0.98	0.96-0.99	**0.01**	0.99	0.97-1.00	0.09
Albumin	0.97	0.92-1.01	0.13	-	-	-
Log_{10} LDH	3.07	1.41-6.66	**0.005**	-	-	-
RMH Score	1.40	1.07-1.83	**0.02**	1.34	1.01-1.77	**0.04**

Table 5: Univariate Cox models for PFS

Variables	Uni HR for PFS	95% CI	p-value
Gender			
Male vs. female	0.99	0.70-1.41	0.97
Treatment			
PD-L1 inhibitor vs. PD-1 inhibitor	0.91	0.47-1.73	0.76
Previous Surgery (yes vs. no)			
Yes	1.17	0.82-1.66	0.38
Previous Radiotherapy (yes vs. no)			
Yes	1.07	0.75-1.52	0.72
Baseline NLR>5			
hNLR vs lNLR	1.14	0.79-1.65	0.48
Age (10 Years)	0.96	0.85-1.09	0.55
Baseline NLR	1.03	0.99-1.06	0.17
Baseline ANC	1.02	0.97-1.08	0.39
Baseline Lymph	0.96	0.69-1.33	0.82
Baseline Hb	**0.99**	0.98-0.99	**0.01**
Albumin	0.95	0.92-0.98	0.005
Log_{10} LDH	0.88	0.48-1.61	0.68
RMH Score	1.09	0.89-1.33	0.41

Table 6: Univariate logistic regression models for any grade and G3/G4 immune toxicity

Variables	Uni OR for any grade	95% CI	p-value	Uni OR for G3/4	95% CI	p-value
Gender						
Male vs. female	0.81	0.43-1.51	0.50	0.78	0.32-1.93	0.60
Treatment						
PD-L1 inhibitor vs. PD-1 inhibitor	0.65	0.21-2.04	0.47	1.08	0.22-5.16	0.93
Previous Surgery (yes vs. no)						
Yes	1.93	1.03-3.64	**0.04**	1.62	0.66-4.00	0.30
Previous Radiotherapy (yes vs. no)						
Yes	1.25	0.67-2.36	0.49	0.59	0.20-1.69	0.33
Baseline NLR>5						
hNLR vs lNLR	0.57	0.29-1.12	0.10	1.18	0.46-3.01	0.73
Age (10 Years)	1.04	0.83-1.30	0.75	1.36	0.91-2.03	0.13
Baseline NLR	0.98	0.91-1.05	0.58	1.06	0.98-1.15	0.13
Baseline ANC	1.05	0.94-1.17	0.42	1.14	1.01-1.30	**0.04**
Baseline Lymph	1.95	1.07-3.54	**0.03**	1.00	0.44-2.27	0.99
Baseline Hb	1.01	0.99-1.03	0.32	1.00	0.97-1.02	0.82
Albumin	1.06	1.00-1.12	0.07	1.02	0.93-1.11	0.73
Log_{10} LDH	0.89	0.35-2.31	0.82	0.72	0.17-3.00	0.65
RMH Score	0.64	0.45-0.92	**0.02**	0.89	0.55-1.47	0.66

Figure 1: Kaplan-Meier survival plots for OS and PFS

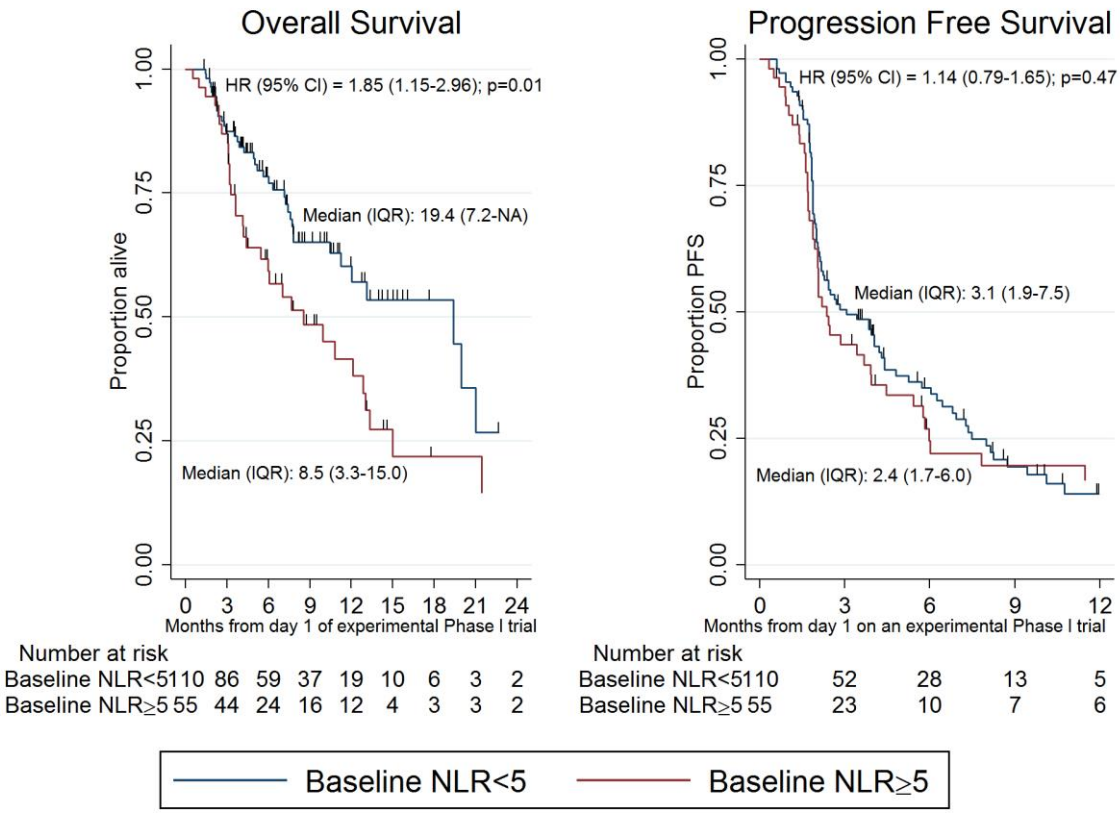

Figure 2: Longitudinal plot of natural log of NLR over time by RECIST response status

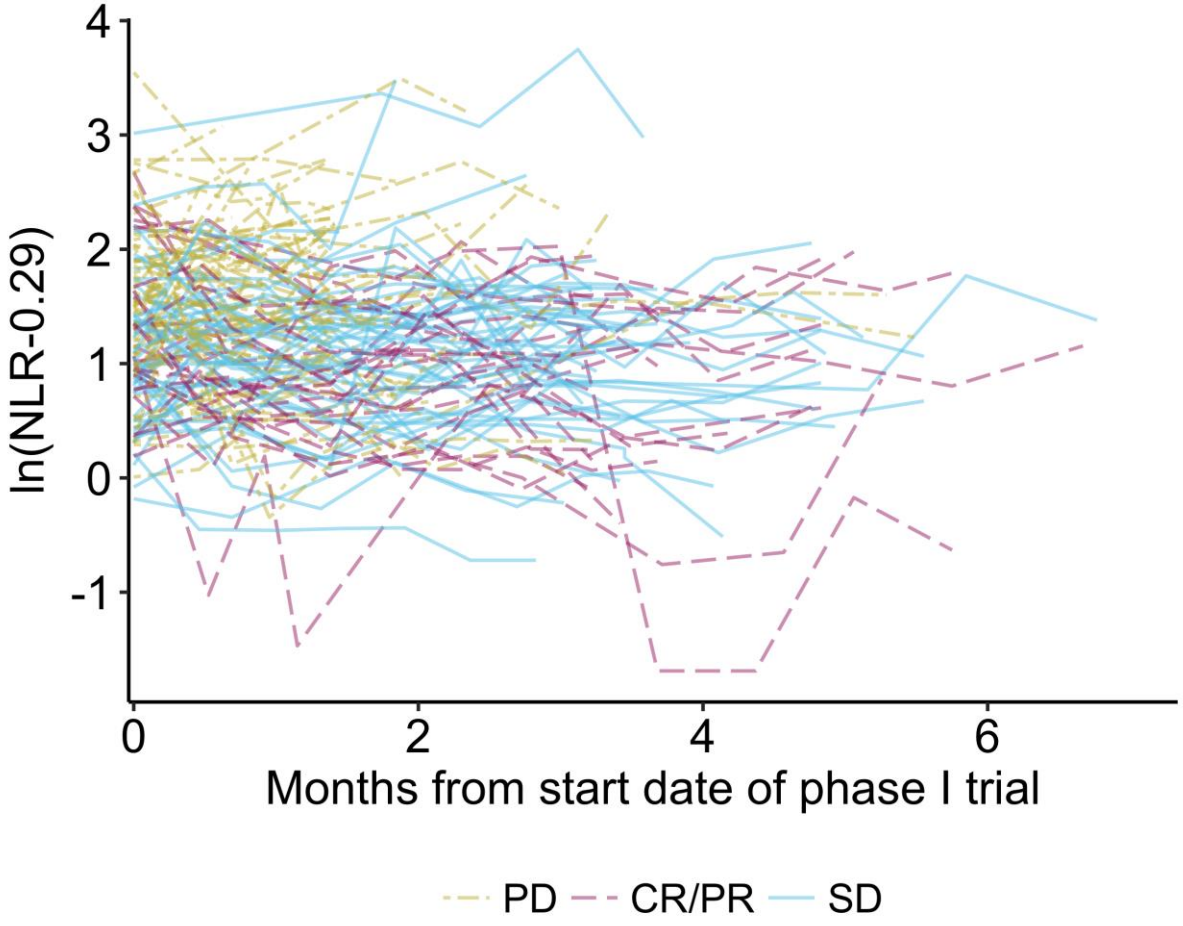

Figure 3: Predicted NLR over time according to RECIST response status