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Running title: Neutrophil-lymphocyte ratio in pancreatic cancer

Paul McLellan[1,2] MD, Julie Henriques[3,4] PhD, Feryel Ksontini[5] MD, Solène Doat[2] MD, Pascal Hammel[6] PhD, Jérome Desrame[7] PhD, Isabelle Trouilloud[8] MD, Christophe Louvet[9] MD, Daniel Pietrasz[10] MD, Dewi Vernerey[3,4] PhD, Jean-Baptiste Bachet[1,2] PhD

1. Sorbonne Université, 4 place Jussieu, 75005, Paris, France
2. Department of Hepato-Gastroenterology, Pitié-Salpêtrière, 47 boulevard de l'Hôpital 75013, APHP, Paris, France
3. Department of Methodology and Quality of Life Oncology, University Hospital, 3 Boulevard Alexandre Fleming, 25000, Besancon, France
4. Bourgogne Franche-Comté Université, INSERM, EFS BFC, UMR1098, Interactions Hôte-Greffon-Tumeur/Ingénierie Cellulaire et Génique, 8 rue du Docteur Jean-François-Xavier Girod 25020 Besançon, France
5. Department of Oncology, Institute Salah-Azaïz, Boulevard du 9 avril 1938, 1006 Tunis, Tunisia
6. Department of Digestive Oncology, Hôpital Beaujon, 100 boulevard du general Leclerc 92110 Clichy, France
7. Department of Gastroenterology, Hôpital Privé Jean Mermoz, 55 avenue Jean Mermoz 69008, Lyon, France
8. Department of Oncology, Hôpital Saint Antoine, 186 rue du faubourg Saint Antoine, 75012, Paris, France
9. Department of Oncology, Institut Mutualiste Montsouris, 54 avenue Jourdan, 75014 Paris, France

10. Department of Hepato-Pancreato-Biliary Surgery, CHB, Hôpital Paul Brousse, 12 avenue Paul Vaillant Couturier, 94800 Villejuif, France

Correspondence to

Dr. Jean-Baptiste Bachet, Department of Gastroenterology and Digestive Oncology, Pitié-Salpêtrière Hospital, Sorbonne University, 47-83 Boulevard de l'Hôpital, Paris 75013, France; Phone: 331-4216-1041; Fax: 331-4216-1238; E-mail: jean-baptiste.bachet@aphp.fr

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Conflict of interest

JBB is a consultant/advisory board member for Amgen, Celgene, and Merck Serono, has received personal fees from Amgen, Bayer, Celgene, Merck Serono, Roche, Sanofi, Servier and non-financial support from Amgen, Merck Serono, and Roche. PH has received grants from Celgene and Roche; personal fees from Baxalta, Celgene, Ipsen, Lilly, Merck Serono, Novartis, and Pfizer and non-financial support from Celgene, Ipsen, Merck Serono, Novartis, and Pfizer. All other authors declare no conflict of interest.

Ethics approval and consent to participate

All experiments utilizing human samples were approved by the Ethical Committee of Medical Research, Pitié-Salpêtrière Hospital, Sorbonne University. The study protocol was approved by the French ethics committee “Ile de France VI”. All patients provided written informed consent before study enrolment.

This study was performed in accordance with the Declaration of Helsinki.

Abstract

High neutrophil-lymphocyte (NLR) at diagnosis is a marker of poor prognosis in metastatic pancreatic adenocarcinoma. Prognosis role of baseline NLR and its early change and NLR evolution under first-line chemotherapy was determined. We conducted a retrospective study based on one prospective cohort from a single center and a randomized open-label multicenter randomized trial. Two hundred and twelve patients were analyzed. Baseline NLR >5 was an independent poor prognosis biomarker for overall survival (HR=2.01, 95%CI 1.33-3.05; *P*=0.001) and for progression-free survival (HR=1.80, 95%CI 1.23-2.65; *P*=0.0026). According to NLR dynamics (*n*=172), patients with NLR ≤5 on days 1 and 15 had a significantly better prognosis than those with NLR ≤5 on day 1 and >5 on day 15 (HR=2.23, 95%CI 1.18-4.21; *P*=0.013), NLR >5 on day 1 and ≤5 on day 15 (HR=3.25, 95%CI 1.86-5.68; *P*<0.001), and NLR >5 on days 1 and 15 (HR=3.37, 95%CI 1.93-5.90; *P*<0.001). Over time, "bad responder" (progression-free survival <6 months) had a significantly higher mean NLR than "good responder" (group effect *P*<0.0001). Seven in 8 patients with baseline NLR >5 had circulating tumor DNA. We confirm the independent prognostic value of baseline NLR >5 in metastatic pancreatic cancer. NLR evolution is also a prognosis indicator in patients with NLR ≤5.

Keywords: neutrophil to lymphocyte ratio, prognostic, metastatic pancreatic cancer, biomarker

Highlights

- What is already known on this subject?

Neutrophils to lymphocytes ratio (NLR) is known as an independent prognosis marker at diagnosis in metastatic pancreatic cancer. Few data are available about NLR follow up and prognosis under chemotherapy.

- What are the new findings?

Early change in NLR can indicate very low survival.

Overtime, patients with good prognosis have lower NLR.

Association between NLR et circulating tumor DNA may be an interesting prognostic biomarker.

- How might it impact on clinical practice in the foreseeable future?

We would advise practitioners to use high NLR during follow-up and chemotherapy administration as an indicator of severity in order to help therapeutic decisions.

Background

Pancreatic adenocarcinoma (PAC) is a severe disease and median overall survival (OS) in patients with metastatic disease is under 12 months.(1,2) The 1-year OS rate is about 30% and decreases at 7% after 5 years. Latest outcome figures confirm increase in the incident rate and prevalence of PAC in western countries.(3) PAC is the fourth cause of cancer deaths in the United States regardless of gender.(4)

First-line treatment of patients with metastatic PAC is based on chemotherapy such as FOLFIRINOX (oxaliplatin, irinotecan, 5-fluorouracil, and leucovorin) (2) or on the combination of gemcitabine and nab-paclitaxel in those with good Eastern Cooperative Oncology Group Performance Status (ECOG PS).(5,6) In PAC frail patients (ECOG PS >2), unfit to support polychemotherapy, gemcitabine monotherapy or best supportive care are the standard.(7)

Tumour markers such as ECOG PS 2, age > 65 years old, liver metastasis,(8) increase in lactate dehydrogenase (LDH), carcinoembryonic antigen (CEA), and carbohydrate antigen 19-9 (CA 19-9) levels have been defined as poor prognostic factors.(9)

CA 19-9, is currently the only FDA-approved biomarker used in PAC, but it has several limitations including lack of specificity. This protein can be elevated in many situations such as cholestasis or others cancers, and can be normal in patients with Lewis negative genotype, representing about 5%-10% of the white population even in the advanced setting.(10,11)

Systemic inflammation is known to promote cancer and metastasis development.(12,13) The role of inflammation and immune response within the tumour and its microenvironment is discussed.(14) The tumour stroma in PAC seems to play a key role in providing drug resistance to immune participation by antigenic tumoral presentation.

Neutrophils represent the majority of white blood cells and participate to anti-tumoral immunity and metastatic spreading.(15) Several tumour biomarkers for PAC have been evaluated such as neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio, fibrinogen, albumin, and C-reactive protein (CRP).(16–19)

A high NLR has been reported as a poor prognosis marker in PAC,(8) but also in various solid cancers.(20–22) In resectable PAC, it is associated with a higher risk of recurrent metastatic disease and short OS.(16) In patients with metastatic PAC, the results of meta-analysis have demonstrated that high values of NLR at diagnosis, ranging from 2.5 to 5, predict poorer OS.(23) NLR was shown to be a more accurate prognosis marker than the platelet-lymphocyte ratio in PAC resectable tumours.(16)

To our knowledge little is known about NLR changes under chemotherapy. Our objective was to analyse a NLR evolution pattern in patients receiving first-line metastatic PAC treatment and to assess the impact of the NLR dynamic evolution on prognosis in this setting.

METHODS

Patients

We performed a retrospective analysis of patients derived from two different prospective cohorts. The first single-centre cohort consisted of consecutive patients who received first-line metastatic PAC treatment at Pitié-Salpêtrière Hospital (the GHPS cohort) from January 2010 to August 2016. Inclusion criteria were cytological or histologically confirmed PAC, metastatic disease, age ≥18 years, and signed consent for use of clinical and biological information. These patients have been included in a prospective translational study (approved by ethics committee) assessing the prognostic value of circulating tumour DNA (ctDNA).(24) The second cohort consisted of patients included in the French open-label, multicentre, randomized phase II AFUGEM trial (clinicaltrials.gov NCT01964534) comparing gemcitabine and nab-paclitaxel to LV5FU2 and nab-paclitaxel.(6) The study protocol was approved by the French ethics committee “Ile de France VI”. All patients provided written informed consent before study enrolment. Studies protocol were conformed to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008) as reflected in a priori approval by the institution's human research committee.

Data collection

For the GHPS cohort, all clinical, pathological and biological data were collected from patient medical records. These included the following: age at diagnosis, ECOG PS at diagnosis, tumour location, tumour differentiation grade, prior history of surgery, number of metastatic sites and location, dosages at diagnosis of albumin, platelets, CEA, CA 19-9 within 15 days before chemotherapy initiation, chemotherapy data (type of regimen, date of the first and last cycle, reasons for treatment interruption), the date of the last assessment and the date of death.

For the AFUGEM cohort, clinical, pathological, and biological data were prospectively collected in the electronic case report form as previously described.(25)

In the both cohorts, white blood count including neutrophil and lymphocyte count in units/mm^3 were collected every 15 days during the first 2 months of treatment (on days 1, 15, 30, 45, and 60) and on days 120 and 180, or at progression if it occurred before day 180. The laboratory tests were performed within 4 days before chemotherapy. Each patient went to the same laboratory during the follow-up. NLR was calculated by dividing the absolute neutrophils count by the absolute lymphocytes counts as previously described.(16)

Progression was defined radiologically according to the RECIST 1.1 criteria or clinically if stopping treatment due to altered general status or death.

Progression free-survival (PFS) was measured from the first chemotherapy administration to the date of progression or death from any cause, whichever occurred first. OS was defined as the time between first chemotherapy administration and death (all causes). Patients alive were censored at the last follow-up.

Statistical analysis

Patients' characteristics at baseline were compared between the two cohorts and between patients with and without NLR at baseline. Median with interquartile range (IQR) and frequencies with percentage were used to describe continuous and categorical variables, respectively that were compared by the Wilcoxon test and Chi-square tests. The final analysed study population consisted of patients who had NLR baseline data.

Patients were categorized into high NLR at baseline group and a low NLR at baseline group using the restricted cubic spline method to define the optimal cut-off value of baseline NLR. Survival curves and follow-up were estimated using the Kaplan-Meier and reverse Kaplan-Meier methods, respectively, described with median and 95% confidence interval (95% CI) and compared with log-rank test. Hazard ratios (HRs) with 95% CIs were estimated with Cox proportional hazard models. Association between baseline characteristics including NLR at

baseline and survivals was assessed with the univariate Cox model. Variables with *P*-value < 0.1 were investigated in a multivariate analysis with a stepwise selection. The proportional hazards assumption was checked graphically by plotting a log-minus log plot of the survival and the correlation between variables.

In order to assess the dynamic change of NLR under first-line chemotherapy, patients were categorized into four groups according to NLR at baseline (day 1) and on day 15: group 1/ NLR under the cut-off value on days 1 and 15, group 2/ NLR under the cut-off value on day 1 and above the cut-off value on day 15, group 3/ NLR above the cut-off value on day 1 and under the cut-off value on day 15, and group 4/ NLR above the cut-off value on days 1 and 15. Both OS and PFS were assessed in all four groups.

For long term NLR follow-up, two groups of patients were defined: a group of "good responders" with PFS >6 months and a group of "bad responders" with PFS <6 months. Median NLR presented with IQR was compared at each date using the Wilcoxon test. Evolution of NLR over time and across groups was estimated with a repeated measures mixed model. Interaction between groups and time was tested.

All analyses were replicated separately in both cohorts to assess robustness of the results.

The database of the AFUGEM trial and the prospective cohort were locked for analysis on September 2016 and December 2016, respectively.

Next-generation sequencing was used for ctDNA analysis as previously described.(24) An exploratory analysis of the correlation between the presence of ctDNA and NLR was performed. The association between the presence of ctDNA and survivals in patients with low NLR was assessed.

All analyses were performed using SAS version 9.3 software (SAS Institute, Cary NC) and R version 3.4.3 software (R Development Core Team, Vienna, Austria; https://www.r-project.org).

All tests were two-sided and *P* values below 0.05 were considered statistically significant

RESULTS

Population of interest

A total of 259 patients with proven metastatic PAC were selected from the GHPS cohort (*n*=145) and the AFUGEM trial (*n*=114; Figure 1). In the GHPS cohort, patients had significantly more ECOG PS scores of 2 or 3, had more tumours with head location, had more often presented with a prior history of surgery, had more frequently administered adjuvant chemotherapy, had fewer liver metastasis, and had the higher incidence of low CA 19-9 levels (Table A.1). OS and PFS were similar between the two cohorts (Figures A.1 and A.2). We then pooled together the data from two prospective cohorts into a single cohort study (*n*=259).

Of the 259 analysed patients, 212 (81%) had NLR at baseline; 127 (88%) in the GHPS cohort and 85 (75%) in the AFUGEM cohort. Characteristics and survival of patients whose NLR was missing were comparable to those whose NLR was available (Table A.2 and Figures A.3 and A.4). The prognostic value of NLR analysis was performed on data from 212 patients excluding those whose NLR was missing at baseline. In GHPS cohort, ten patients with ECOG PS 3 due to symptoms relative to the disease and without comorbidity began a palliative chemotherapy. These patients were younger than patients with ECOG PS 0-2 (66.8 vs 69.4 years).

Determination of the NLR cut-off value

We used restricted cubic spline method to define the relation between NLR and OS. There was an increased risk of death until NLR was equal to 5 and then stabilization was observed (Figure A.5). We assumed that a baseline NLR value of 5 was a potential cut-off value for metastatic PAC patients. Therefore, this threshold was chosen for subsequent analyses. Overall, 50 (24%) patients had NLR >5 at baseline.

Prognostic value of NLR at baseline

We compared patients with low NLR ≤5 (*n*=162) to those with high NLR >5 (*n*=50) at baseline. The two groups were comparable in terms of sex, tumour differentiation grade, and number of metastatic sites. Patients in the high NLR group had statistically poorer ECOG PS, presented less frequently a history of surgical resection, and had more often lower albumin and increased CEA levels (Table 1). Patients with NLR >5 at baseline had significantly shorter PFS (median PFS 2.1 months, 95% CI 1.6-3.4 versus 7.2 months, 95% CI 5.4-8.2; *P*<0.0001) and OS (median OS 3.3 months, 95% CI 2.2-5.2 versus 13.8 months, 95% CI 11.0-16.6; *P*<0.0001) than those with NLR ≤5 (Table 1; Figures A.6 and A.7). Results were unchanged after exclusion of the 10 patients with ECOG PS 3 at baseline (Figures A.8 and A.9). Among patients with ECOG PS 3 at baseline, the 6 patients with a NLR >5 died before two months whereas 2 of the 4 patients with a NLR ≤5 were alive at 6 months.

Univariate and multivariate analysis for OS and PFS at baseline

In univariate analysis, poor prognosis factors for OS were ECOG PS 2-3 (*P*<0.0001), body and tail tumour location (*P*=0.0022), age ≥65 years (*P*=0.02), more than three metastatic sites (*P*=0.0066), CEA ≥8 (*P*=0.0018), CA 19-9 ≥1000 UI/ml (*P*<0.001), and NLR >5 (*P*<0.0001). Factors associated with better prognosis were previous history of primary tumour resection (*P*=0.0012), well-differentiated tumour (*P*=0.001), and albumin level ≥40 g/L (*P*=0.0005; Table 2).

In multivariate analysis, NLR >5 at baseline was an independent poor prognosis biomarker for OS (HR=2.01, 95% CI 1.33-3.05; *P*=0.001; Table 2) and for PFS (HR=1.80, 95% CI 1.23-2.65; *P*=0.0026; Tables A.3 and A.4).

Prognostic value of early change of NLR

NLR data at baseline (day 1) and on day 15 were available for 171 patients. Patients with NLR ≤5 on day 1 and on day 15 (*n*=125) had significantly better prognosis compared to those

with NLR >5 at one or two dates (Figure 2). Consistent results were found for PFS (Figure A.10). Patients with the worst prognostic were those with NLR >5 at baseline and on day 15. Results were unchanged after exclusion of the 10 patients with ECOG PS 3 at baseline (Figures A.11 and A.12).

NLR evolution over time

NLR differences were analysed at each date in the group of "good responders" with PFS >6 months (*n*=82) and in the group of "bad responders" (*n*=121). "Bad responders" had a significantly higher median NLR at all dates than "good responders", except that on day 45. The evolution of NLR over time showed a significant group effect ($P<0.0001$), no significant time effect ($P=0.1031$), and nor interaction between both groups ($P=0.1252$; Table 3 and Figure 3).

Analyses in each study cohort

Analyses were replicated in both cohorts separately (Tables A.5 to A.8). Patients with NLR ≤5 at baseline and on day 15 had longer survival than other patients in both cohorts (Figure A.13 to A.17). Results were unchanged when analysed in the fluoropyrimidine plus nab-paclitaxel arm of the AFUGEM trial alone (data not shown).

NLR correlation with ctDNA

ctDNA data before first-line chemotherapy was available for 52 patients in the GHPS cohort. The ctDNA was more often detected in patients with NLR>5 at baseline (7/8, 87%) than in those with NLR ≤5 (22/40, 55%). In patients with NLR ≤5, the presence of ctDNA was associated with shorter PFS and OS (Figure A.18 and A.19).

DISCUSSION

In this study, we confirmed that high NLR (>5) at baseline is an independent prognostic biomarker of OS and PFS in patients treated in first-line for metastatic PAC. Moreover, NLR dynamic during the first 15 days of treatment also appears to be a prognostic biomarker in patients with NLR ≤ 5. Depending on PFS, the mean NLR was higher at each date for patients with poor prognosis. Therefore, NLR increasing over time appears to be a prognostic biomarker.

High NLR is known to be associated with poor OS in various solid cancers such as lung,(20) breast cancer,(21) or ovarian.(22) In 2014, a meta-analysis of 100 studies comprising more than 40 000 patients confirmed the prognosis role of high NLR in gastro-oesophageal cancers, cholangiocarcinoma, hepatocellular cancer, colorectal cancer, renal cell carcinoma, and non-small cell lung cancer.(26) In PAC, NLR is also useful in patients with advanced PAC treated by chemoradiotherapy alone (27) or in those receiving chemoradiotherapy before curative surgery.(28)

The optimal NLR cut-off value of 5 in our study is consistent with that of previous studies.(5) Nevertheless, in PAC, several NLR thresholds have been reported. In a recent meta-analysis the cut-off values for elevated NLR were not consistent and ranged from 2.5 to 4, so it did not provide the most optimal value to be used.(23)

In our study, low ECOG PS and albumin, and high CEA levels were correlated with high NLR. These factors are known to be associated with poor prognosis in metastatic PAC. Systemic inflammation, reflected by high NLR, could emphasizes patients' symptoms such as anorexia and asthenia, and, consecutively, be responsible of a poorer PS. NLR was lower in patients who had curative intent resection. These patients undergo regular follow-up visits after surgery. The tumor burden and the systemic inflammation associated are thus probably less important at relapse diagnosis than in patients with metastasis at diagnosis. The step-wise multivariate analysis strategy confirmed the independent poor prognosis value of baseline high NLR. Other systemic biomarkers such as CRP, albumin, platelet-lymphocyte ratio may

316 also give indication about the immune response of the host. Among them, high NLR seems to
317 be the most accurate.(19)
318 NLR is affordable and easily accessible biological marker. Various cytokines like interferon
319 or interleukine-6, and angiogenic factors (e.g. platelets derived growth factors) are the factors
320 of interest in evaluating prognosis of patients with PAC,(17,29) though none of these is
321 currently recommended in clinical practice. Other inflammatory markers such as the Glasgow
322 prognostic score based on albumin and CRP or the NARCA prognosis score based on
323 neutrophils-to-albumin ratio and CA 19-9 have been also proposed.(30) Based on the first
324 international consensus on mandatory baseline and prognostic characteristics in future trials
325 for the treatment of unresectable PAC reported by Ter Veer et al., CRP and NLR were
326 defined as the compulsory measurements.(31)
327 The prognostic potential of the systemic inflammation-based markers in PAC is still unclear.
328 PAC is known for high inflammation not only in the tumour's stroma and microenvironment,
329 but also on a systemic level. We may hypothesize that the severity of systemic response
330 reflects aggressiveness of the tumour microenvironment. An elevated NLR may originate
331 from raised neutrophil or decreased lymphocyte counts. PAC microenvironment was proven
332 to induce tumour-associated neutrophils, which promotes metastatic invasion.(32)
333 Neutrophils can induce angiogenesis and suppress anti-tumour activity as such allowing
334 tumour growth. They also produce or release various chemokines (including VEGF),
335 metalloprotease, and reactive oxygen species that play a key role in tumour vascular
336 development and migration.(33–35) The activation of the *KRAS* pathway, frequent in
337 PAC,(36) was shown to recruit and activate neutrophils.(37) Transforming growth factor-beta
338 in the tumour stroma was shown to induce specific neutrophils with pro-tumour
339 phenotype.(38)
340 Decreased lymphocyte counts resulting in raised NLR may explain weaker defences against
341 the tumour and the cancer ability to escape chemotherapy response. Decreased lymphocyte
342 counts has been reported to be associated with shorter survival in PAC.(39)

High NLR at baseline has been reported as a strong independent prognostic biomarker, but its evolution over time could be also of interest. Chen et al. assessed NLR evolution between baseline and after 2 cycles of chemotherapy in 132 patients treated for advanced or metastatic PAC.(40) The value of 2.78 was selected as the NLR cut-off. Patients with increased NLR at 1 month had a poorer prognostic than others. We performed the same analysis in our population, but did not find any difference (Table A.12). However, the four groups strategy used in our work emphasizes the poor prognosis associated with a high NLR level whatever the moment. Chen et al reported relatively similar data with their methodology, in particular by defining their four sub-groups based on delta. Others studies are necessary to define the best method to use in clinical practice (delta of NLR or threshold at 5). We hypothesized that patients with high NLR at baseline turning <5 on day 15 would have an intermediate prognosis, but we did not observe it.

The presence of ctDNA is a prognostic biomarker at baseline in PAC as in other solid tumours.(24) Our exploratory results suggest an association between high NLR and presence of ctDNA, though one patient with NLR >5 did not have detectable ctDNA. Moreover, the presence of ctDNA seemed to be a prognostic biomarker in patients with NLR <5 at baseline. These two biomarkers may provide different information. NLR can reflect more the state of the inflammation and immunodepression associated to the disease whereas the ctDNA can be more correlated with the "aggressiveness" of the tumour cells or the tumour burden. In order to better understanding these points more data are necessary.

The retrospective design, the use of different chemotherapy regimens in first-line (Tables A.9 to A.11), the lack of data regarding corticosteroids use and granulocyte colony-stimulating factor administration, and the relative low number of patients in each subgroup for NLR dynamic analyses, especially in NLR >5 group, are limitations to our study. To explore potential biases, the NLR analyses were done for patients with and without NLR information at baseline and for each cohort in order to detect the subgroup effect. These analyses showed that NLR at baseline and its evolution under treatment are comparable between each cohort.

Therefore, the study patients are representative of the general population of patients with metastatic PAC receiving first-line chemotherapy in France. Given the heterogeneity of chemotherapy regimens, we did not analyse the relation between NLR and treatment toxicity. ctDNA data were only available for a subgroup of patients and these results are of an exploratory nature.

The aim of assessing affordable, easily accessible, and performant biomarkers remains a key to treatment optimization, combined with clinical and imaging features. With these objectives, NLR appears as a promising dynamic and prognostic biomarker.

In conclusion, high NLR before or during chemotherapy was indicative of a poor prognosis in patients with metastatic PAC. These results suggest the potential interest of following NLR at each chemotherapy cycle. Further validation in prospective studies is required.

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Table and Figure Legends

Table 1. Comparison of characteristics between patients according to NLR at baseline

Table 2. Univariate and multivariate analyses of the association between baseline patient characteristics and overall survival

Table 3. Description of NLR over time according to progression-free survival under first-line chemotherapy (6<months or >6 months) (A) and mixed model of repeated measures and time as categorical variable (from day 1 to day 180) (B)

Figure 1. Population flow chart

Figure 2. Overall survival curves according to NLR at baseline and on day 15

Figure 3. Evolution curves of NLR from day 1 to day 180 under first-line of chemotherapy in "good responders" (PFS >6 months) and "bad responders" (PFS <6 months) (*n*=212)

Abbreviations

ECOG PS: Eastern Cooperative Oncology Group Performance Status

CA 19-9: carbohydrate antigen 19-9

CEA: carcinoembryonic antigen

NLR: neutrophil to lymphocyte ratio

OS: overall survival

PFS: progression-free survival

HR: hazard ratio

CI: confidence interval

IQR: interquartile range

GHPS: Pitié-Salpêtrière Hospital Group

Characteristics		NLR available *N* = 212 *n* (%)	NLR ≤ 5 *N* = 162 *n* (%)	NLR > 5 *N* = 50 *n* (%)	*P*-value
Age*	Median	65.8	65.3	67.9	0.059
	IQR	60.3-73.0	58.9-72.7	62.5-73.8	
	Missing	9	4	5	
Gender**	Male	131 (62)	103 (64)	28 (56)	0.3349
	Female	81 (38)	59 (36)	22 (44)	
ECOG PS**	0	67 (32)	59 (36)	8 (16)	0.0002
	1	83 (39)	67 (41)	16 (32)	
	2	52 (24)	32 (20)	20 (40)	
	3	10 (5)	4 (2)	6 (12)	
Primary tumour location**	Head	107 (50)	85 (53)	21 (42)	0.1762
	Body	38 (18)	26 (16)	12 (24)	
	Tail	44 (21)	30 (18)	14 (28)	
	Head and body	15 (7)	14 (9)	1 (2)	
	Body and tail	8 (4)	6 (4)	2 (4)	
Stage at diagnosis**	I/II	40 (19)	36 (22)	4 (8)	0.0796
	III	7 (3)	5 (3)	2 (4)	
	IV	165 (78)	121 (75)	44 (88)	
Tumour differentiation	Well	64 (37)	52 (38)	12 (33)	0.6109
grade	Moderate	85 (49)	68 (49)	17 (47)	
	Poor	25 (14)	18 (13)	7 (19)	
	Missing	38	24	14	
Number of metastatic sites	1	131 (62)	104 (65)	27 (54)	0.1611
	≥2	79 (38)	56 (35)	23 (46)	
	Missing	2	2	0	
Liver metastases**		138 (65)	103 (64)	35 (70)	0.4051
Resection of primary tumour**		59 (28)	52 (32)	7 (14)	0.0126
Adjuvant chemotherapy					0.062
	Yes	40 (19)	35 (21)	5 (10)	
	Missing	2	2	0	
Albumin (g/L)	Median	37	38	34	0.0341
	IQR	32-41	34-41	30-40	
	Missing	11	8	3	
CA 19-9 (UI/ml)	Median	450	390	2143	0.09
	IQR	37-3616	39.90-1831.5	31-10000	
	Missing	15	10	5	
CEA (ng/ml)	Median	5	4	8.8	0.0308
	IQR	2-19	2-17	3-32	
	Missing	17	11	6	
Cohort*	AFUGEM	85 (40)	64 (39)	21 (42)	0.7531
	GHPS	127 (60)	98 (60)	29 (58)	
Death		159 (77)	112 (71)	47 (94)	0.0009
Progression		120 (57)	98 (60)	22 (44)	0.0397
OS median (95% CI)		10.7 (8.9-13.3)	13.8 (11.0-16.6)	3.3 (2.2-5.2)	< 0.0001
PFS median (95% CI)		5.4 (4.4-6.2)	7.2 (5.4-8.2)	2.1 (1.6-3.4)	< 0.0001

Table 1. Comparison of characteristics between patients according to NLR at baseline

Abbreviations: NLR=neutrophil to lymphocyte ratio; ECOG PS=Eastern Cooperative Oncology Group Performance Status; CA 19-9=carbohydrate antigen 19-9; CEA=carcinoembryonic antigen; IQR=interquartile range; OS=overall survival; PFS=progression-free survival

*Age at randomization for AFUGEM and age at first-line chemotherapy initiation for retrospective study

**no missing data

Table 2. Univariate and multivariate analyses of the association between baseline patient characteristics and overall survival

		Univariate analysis				Multivariate analysis *N* = 186; *N* events = 133		
		***N* (events)**	**HR**	**95% CI**	***P*-value**	**HR**	**95% CI**	***P*-value**
Age	< 65 years	90 (63)	1		0.0208			
	≥ 65 years	113 (88)	1.47	1.06-2.04				
Gender	Male	131 (99)	1		0.1338			
	Female	81 (52)	0.77	0.55-1.08				
ECOG PS	0-1	150 (103)	1		< 0.0001	1		0.0002
	2-3	62 (48)	2.85	2-4.05		2.32	1.48-3.62	
Primary tumour location	Head/ head and body	122 (82)	1		0.0022	1		0.0086
	Other	90 (69)	1.66	1.20-2.30		1.66	1.14-2.43	
Differentiation grade	Poor and moderate	110 (86)	1		0.001			
	Well	64 (39)	0.46	0.31-0.69				
Resection of primary tumour	No	153 (111)	1		0.0012	1		0.0133
	Yes	59 (40)	0.54	0.37-0.78		0.57	0.37-0.89	
Number of metastatic sites	1	131 (93)	1		0.0066	1		0.0353
	2	58 (41)	1.02	0.71-1.48		1.23	0.82-1.84	
	≥ 3	21 (16)	2.37	1.38-4.06		2.19	1.20-4.01	
Albumin (g/l)	< 40	132 (101)	1		0.0005	1		0.0006
	≥ 40	69 (43)	0.53	0.37-0.76		0.48	0.31-0.73	
CA 19-9 (UI/ml)	< 1000	120 (79)	1		< 0.0001	1		0.0206
	≥ 1000	77 (62)	2.16	1.53-3.06		1.57	1.07-2.30	
CEA (ng/ml)	< 8	115 (84)	1		0.0118			
	≥ 8	80 (57)	1.55	1.10-2.17				
NLR at baseline	≤ 5	162 (109)	1		< 0.0001	1		0.001
	> 5	50 (42)	3.22	2.23-4.64		2.01	1.33-3.05	

Abbreviations: HR=hazard ratio; NLR=neutrophil to lymphocyte ratio; ECOG PS=Eastern Cooperative Oncology Group Performance Status; CA 19-9=carbohydrate antigen 19-9; CEA=carcinoembryonic antigen; IQR=interquartile range

Table 3. Description of NLR over time according to progression-free survival under first-line chemotherapy (< 6 months or > 6 months) and results of mixed model of repeated measures and time as categorical variable (from day 1 to day 180)

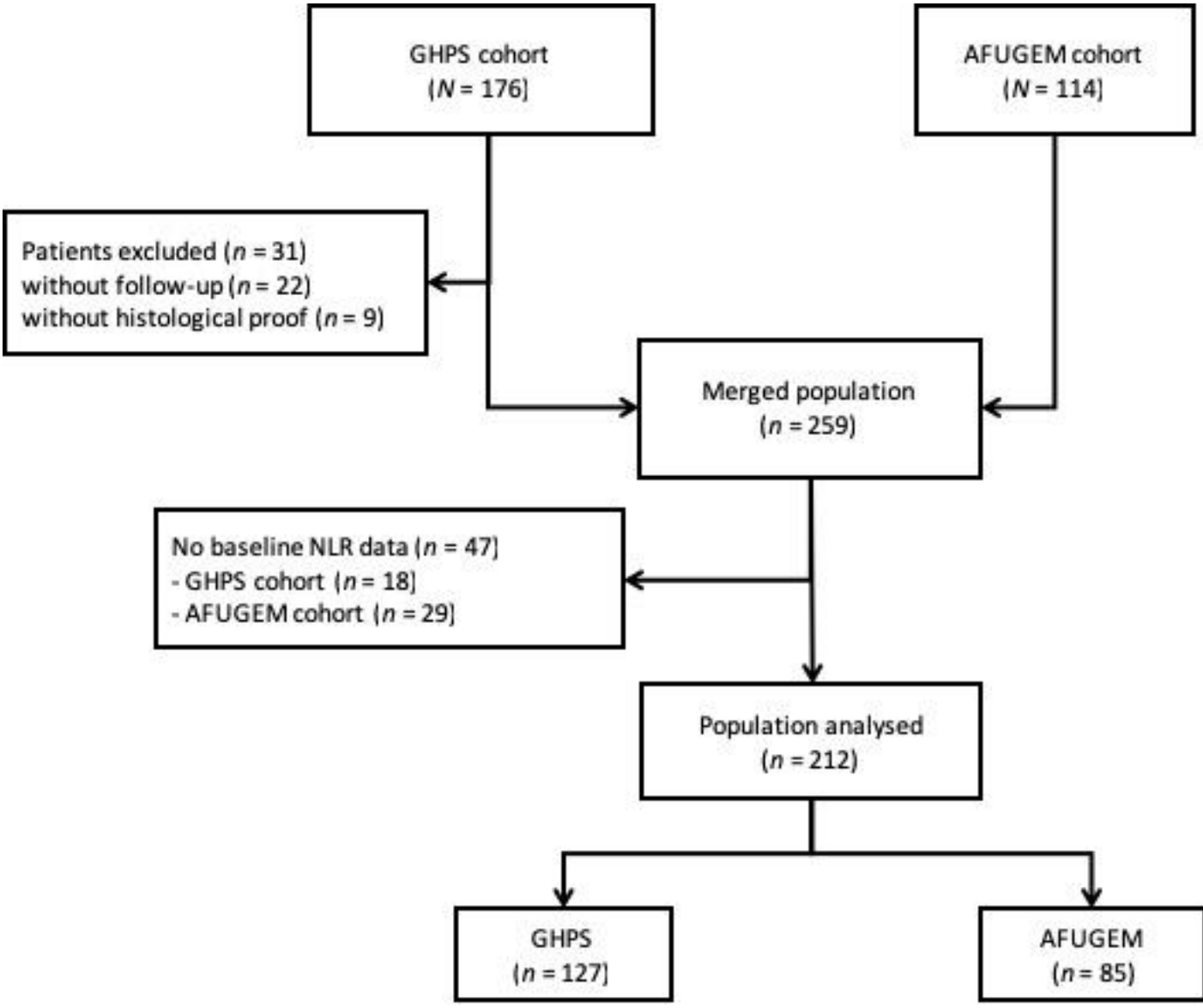
Description of NLR over time according to progression-free survival

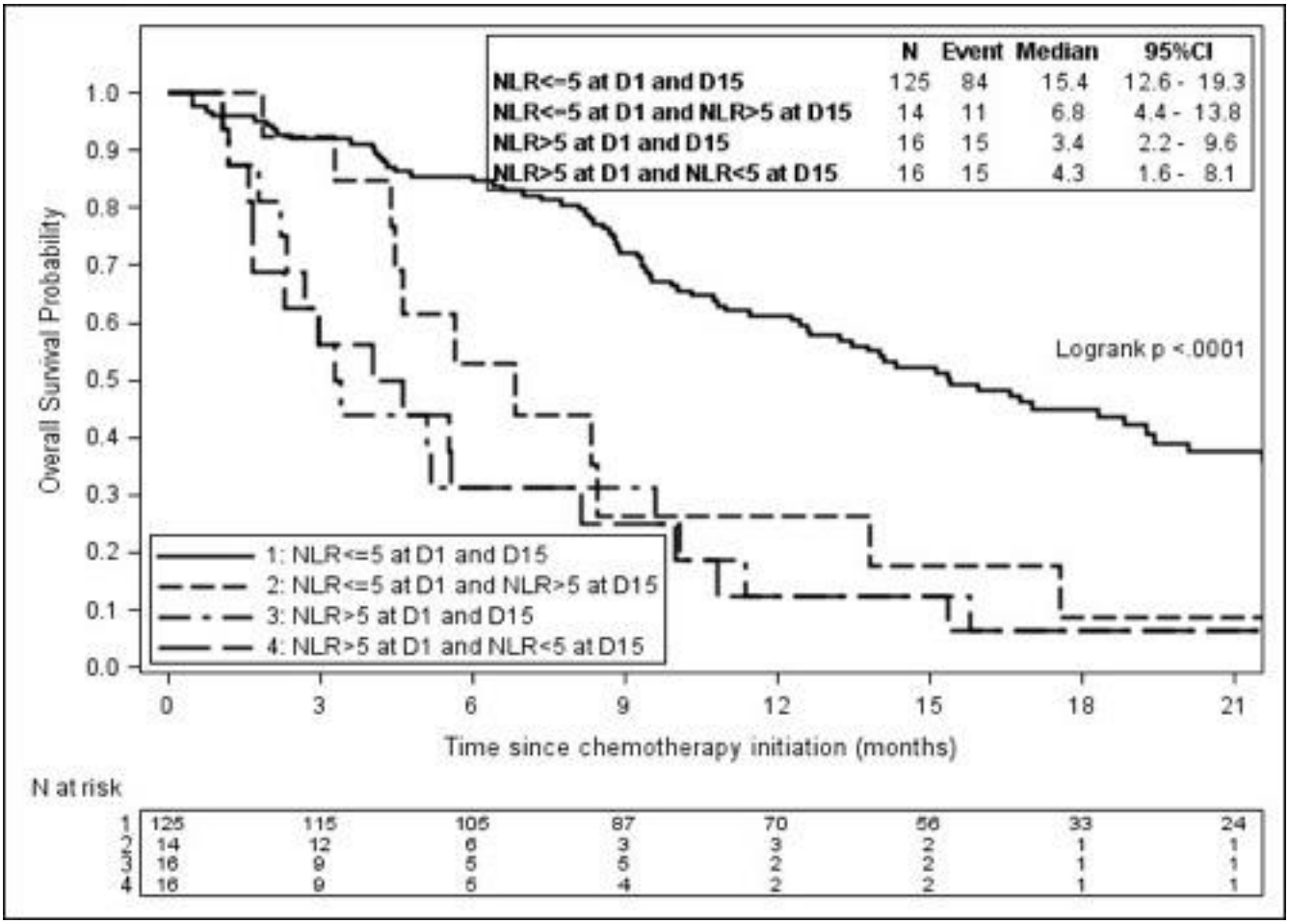
Under first-line chemotherapy (<6 months or >6 months)

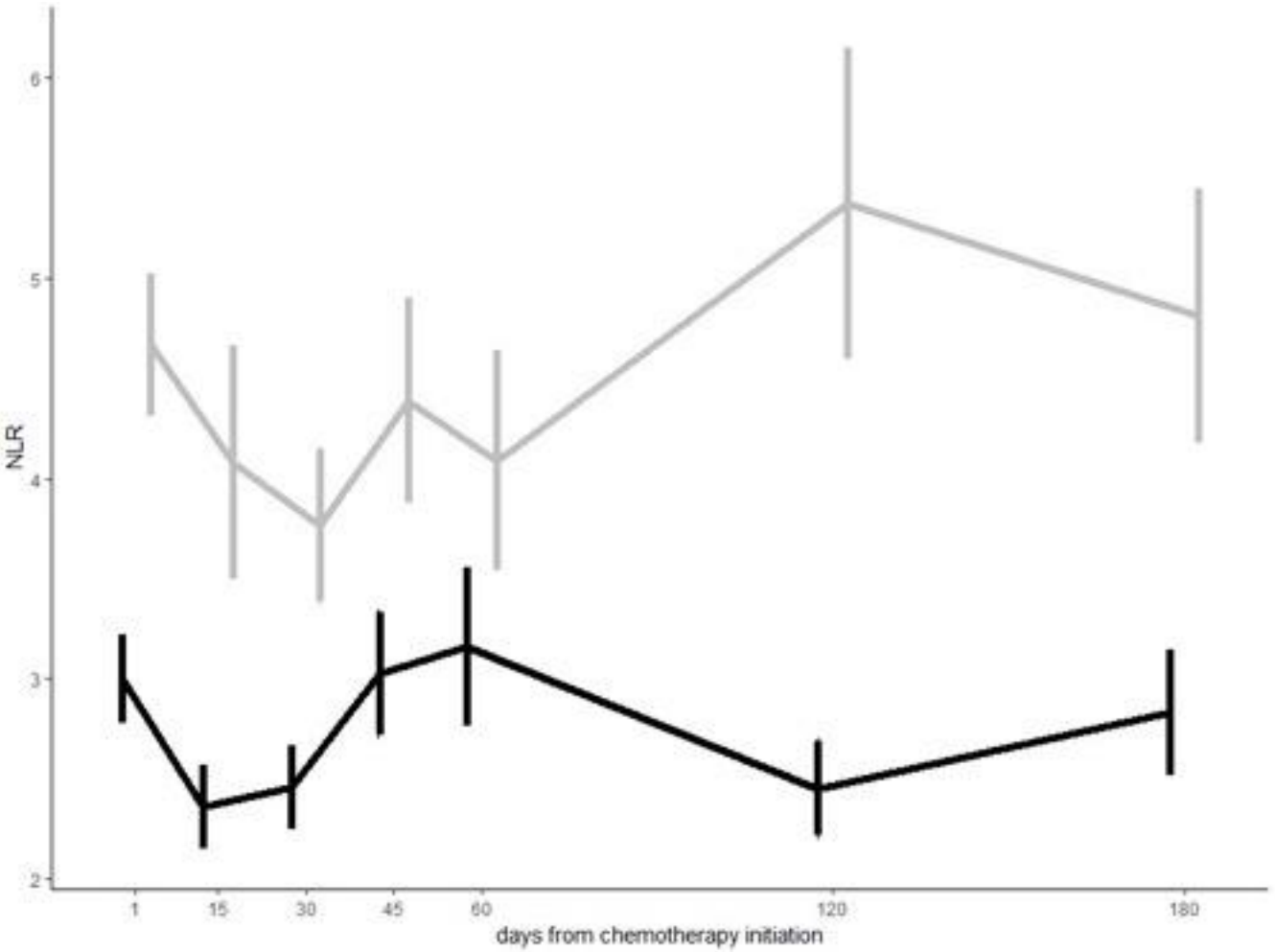
	Responder	***N***	**Mean**	**SD**	**Median**	**IQR**
D1	bad	129	4.67	4.04	3.80	2.0-6.20
	good	83	3.00	2.02	2.69	1.75-3.49
D15	bad	98	4.08	5.76	2.63	1.28-4.81
	good	74	2.36	1.82	1.82	1.22-2.88
D30	bad	83	3.77	3.48	2.69	1.47-5.13
	good	75	2.45	1.81	1.89	1.45-3.17
D45	bad	85	4.39	4.72	2.76	1.53-5.31
	good	79	3.02	2.74	2.29	1.31-3.32
D60	bad	58	4.09	4.18	2.83	1.79-4.25
	good	74	3.16	3.42	2.11	1.18-3.28
D120	bad	31	5.37	4.35	3.30	2.45-7.25
	good	66	2.45	1.92	2.05	1.28-2.95
D180	bad	15	4.81	2.47	5.53	2.53-6.23
	good	59	2.83	2.40	1.98	1.32-3.29

Results of mixed model of repeated measures and time as categorical variable (from day 1 to day 180)

Effect	***N*** **DF**	**Den DF**	**F Value**	**Pr > F**
Good responder	1	210	34.69	< 0.0001
Evaluation	6	785	1.77	0.1031
Responder*evaluation	6	785	1.67	0.1252


GHPS cohort
(N = 176)
AFUGEM cohort
(N = 114)
Patients excluded (n = 31)
without follow-up (n = 22)
without histological proof (n = 9)
Merged population
(n = 259)
No baseline NLR data (n = 47)
- GHPS cohort (n = 18)
- AFUGEM cohort (n = 29)
Population analysed
(n = 212)
GHPS
(n = 127)
AFUGEM
(n = 85)


N Event Median 95%CI
NLR<=5 at D1 and D15 125 84 15.4 12.6 - 19.3
NLR<=5 at D1 and NLR>5 at D15 14 11 6.8 4.4 - 13.8
NLR>5 at D1 and D15 16 15 3.4 2.2 - 9.6
NLR>5 at D1 and NLR<5 at D15 16 15 4.3 1.6 - 8.1
Logrank p <.0001
Overall Survival Probability
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1: NLR<=5 at D1 and D15
2: NLR<=5 at D1 and NLR>5 at D15
3: NLR>5 at D1 and D15
4: NLR>5 at D1 and NLR<5 at D15
0 3 6 9 12 15 18 21
Time since chemotherapy initiation (months)
N at risk
1 125 115 105 87 70 56 33 24
2 14 12 6 3 3 2 1 1
3 16 9 5 5 2 2 1 1
4 16 9 5 4 2 2 1 1


NLR
6
5
4
3
2
1
15
30
45
60
120
180
days from chemotherapy initiation
Res

APPENDICES

Paul McLellan, Sorbonne Université, Paris, France; Department of Hepato-Gastroenterology, Groupe Hospitalier Pitié Salpêtrière, Paris; France. Email: paul.mclellan@hotmail.fr

Jean Baptiste Bachet, Sorbonne Université, Paris, France; Department of Hepato-Gastroenterology, Groupe Hospitalier Pitié Salpêtrière, Paris; France. Email: jean-baptiste.bachet@aphp.fr

Abbreviations

ECOG PS: Eastern Cooperative Oncology Group Performance Status
CA 19-9: Carbohydrate antigen 19-9
CEA: Carcinoembryonic antigen
NLR: Neutrophil to lymphocyte ratio
OS: Overall survival
PFS: Progression-free survival
HR: Hazard ratio
CI: Confidence interval
GHPS: Pitié-Salpêtrière Hospital Group
IQR: Interquartile range

Figure A19. Progression-free survival in patients with baseline NLR < 5 according to the presence of ctDNA in the GHPS cohort

Table A1. Comparison of baseline characteristics between the two cohorts of patients

Characteristics		**Total** ***N*=259** ***n* %**	**GHPS** ***N*=145** ***n* %**	**AFUGEM** ***N*=114** ***n* %**	***P*-value**
Age					0.904
	Median	65.7	65.4	66.1	
	IQR	60.6-72.8	60.4-72.9	61.5-72.7	
	Missing	16	16	0	
Gender*					0.8185
	Male	157 (60.6)	87 (60.0)	70 (61.4)	
	Female	102 (39.4)	58 (40.0)	44 (38.6)	
ECOG PS*					
	0	78 (30.1)	41 (28.3)	37 (32.5)	0.0002
	1	109 (42.1)	50 (34.5)	59 (51.8)	
	2	59 (22.8)	41 (28.3)	18 (15.8)	
	3	13 (5.0)	13 (9.0)	0	
Tumour location					< 0.0001
	Head	123 (47.7)	80 (55.2)	43 (38.1)	
	Body	47 (18.2)	22 (15.2)	25 (22.1)	
	Tail	53 (20.5)	29 (20)	24 (21.2)	
	Head and body	21 (8.1)	14 (9.7)	7 (6.2)	
	Body and tail	14 (5.4)	0	14 (12.4)	
	Missing	1	0	1	
Stage*					< 0.0001
	I/II	43 (16.6)	38 (26.2)	5 (4.4)	
	III	7 (2.7)	5 (3.5)	2 (1.8)	
	IV	209 (80.7)	102 (70.3)	107 (93.9)	
Differentiation grade					0.3942

	Well	74 (35.8)	51 (38.1)	23 (31.5)	
	Moderate	103 (49.8)	62 (46.3)	41 (56.2)	
	Poor	30 (14.5)	21 (15.7)	9 (12.3)	
	Missing	55	11	41	
Number of metastatic sites					0.4714
	1	156 (60.7)	84 (58.7)	72 (63.2)	
	≥ 2	101 (39.3)	59 (41.3)	42 (36.8)	
	Missing	2	2	0	
Liver metastases*					0.0001
		162 (62.6)	76 (52.4)	86 (75.4)	
Resection of primary tumour*					0.0001
		64 (24.7)	49 (33.8)	15 (13.2)	
Adjuvant chemotherapy					< 0.0001
	Yes	44 (17.2)	39 (26.9)	5 (4.5)	
	Missing	3	0	3	
Albumin (g/l)					0.0021
	Median	37	36	38.2	
	IQR	32-40.5	30-39	34-42	
	Missing	15	15	0	
CA 19-9 (UI/ml)					0.0093
	Median	496	355	891	
	IQR	39.8-4413.0	29-2555.5	65-9205	
	Missing	18	9	9	
CEA (ng/ml)					0.1558
	Median	5.4	4.5	6.0	
	IQR	2.3-19.6	2-18	2.5-23	
	Missing	20	9	11	
NLR at baseline					0.9845
	Median	2.9	2.8	3.0	
	IQR	1.9-4.9	1.9-4.9	1.9-4.9	

	Missing	47	18	29	
Death		182 (70.3)	103 (71.0)	79 (73.8)	0.7615
Progression		145 (56.0)	83 (57.2)	62 (54.4)	0.6458
OS in months					
	Median 95% CI	10.32 (9.3-12.6)	10.32 (8.4-13.8)	10.02 (8.8-13.6)	0.8061
PFS in months					
	Median 95% CI	5.29 (4.4-6.1)	4.57 (3.3-5.6)	6.41 (4.8-7.7)	0.8121
Follow-up in months					
	Median 95% CI	19.35 (17.3-23.6)	31.38 (24.4-61.6)	16.89 (15.5-17.9)	0.0119

*no missing data

Abbreviations: IQR=interquartile range; ECOG PS=Eastern Cooperative Oncology Group Performance Status; CA 19-9=carbohydrate antigen; CEA=Carcinoembryonic antigen; NLR=neutrophil-to-lymphocyte ratio; OS=overall survival; PFS=progression-free survival

Table A2. Comparison of characteristics between patients with and without NLR data at baseline

Characteristics	**Total**	**no NLR data at baseline**	**NLR data at baseline**	***P*-value**
	***N*=259** ***n* %**	***N*=47** ***n* %**	***N*=212** ***n* %**	
Age				0.1042
Median	65.7	65.3	65.8	
IQR	60.6-72.8	63.4-70.3	60.3-73.0	
Missing	16	7	9	
Gender*				0.4112
Male	157 (60.6)	26 (5.3)	131 (61.8)	
Female	102 (39.4)	21 (44.7)	81 (38.2)	
ECOG PS*				0.1697
0	78 (30.1)	11 (23.4)	67 (31.6)	
1	109 (42.1)	26 (55.3)	83 (39.2)	
2	59 (22.8)	7 (14.9)	52 (24.5)	
3	13 (5)	3 (6.4)	10 (4.7)	
Tumour location				0.0463
Head	123 (47.7)	16 (34.8)	107 (50.5)	
Body	47 (18.2)	9 (19.6)	38 (17.9)	
Tail	53 (20.1)	9 (19.6)	44 (20.8)	
Head and body	21 (8.1)	6 (13.0)	15 (7.1)	
Body and tail	14 (5.4)	6 (13.0)	8 (3.8)	
Missing	1	1	0	
Stage*				0.0418
I/II	43 (16.0)	3 (6.4)	40 (18.9)	
III	7 (2.7)	0 (0)	7 (3.3)	
IV	209 (80.7)	44 (93.6)	165 (77.8)	
Differentiation grade				0.7717
Well	74 (35.8)	10 (30.3)	64 (36.8)	

Moderate	103 (49.8)	18 (54.6)	85 (48.9)	
Poor	30 (14.5)	5 (15.2)	25 (14.4)	
Missing	55	14	38	
Number of metastatic sites				0.2436
1	156 (60.7)	25 (53.2)	131 (62.4)	
≥ 2	101 (39.3)	22 (46.8)	79 (37.6)	
Missing	2	0	2	
Liver metastases*	162 (62.6)	24 (51.1)	138 (65.1)	0.0722
Resection of primary tumour*	64 (24.7)	5 (10.7)	59 (27.8)	0.0134
Adjuvant chemotherapy				0.0919
Yes	44 (17.2)	4 (8.7)	40 (19.1)	
Missing	3	1	2	
Albumin (g/l)				0.1042
Median	37	35	37	
IQR	32-40.5	30.4-39	32-41	
Missing	15	4	11	
CA 19-9 (UI/ml)				0.161
Median	496	968.2	450	
IQR	39.8-4413.0	64.8-14000	37-3616	
Missing	18	3	15	
CEA (ng/ml)				0.1421
Median	5.4	8.8	5	
IQR	2.3-19.6	2.7-45.3	2-19	
Missing	20	3	17	
Cohort*				0.0069
AFUGEM	114 (44.0)	29 (61.7)	85 (40.1)	

GHPS	145 (55.9)	18 (38.3)	127 (59.9)	
Death	182 (70.3)	31 (66.0)	151 (71.2)	0.4746
Progression	145 (56.0)	25 (53.2)	120 (56.6)	0.6698
OS in months				
Median 95% CI	10.32 (9.3-12.6)	9.89 (6.7-15.3)	10.74 (8.9-13.3)	0.7894
PFS in months				
Median 95% CI	5.29 (4.4-6.1)	4.37 (2.3-7.1)	5.36 (4.4-6.2)	0.7104
Follow-up in months				
Median 95% CI	19.35 (17.3-23.6)	17.28 (15.5-30.7)	19.35 (17.4-24.6)	0.1845

*No missing data

Abbreviations: IQR= interquartile range; ECOG PS=Eastern Cooperative Oncology Group Performance Status; CA 19-9=carbohydrate antigen; CEA=carcinoembryonic antigen; NLR=neutrophil-to-lymphocyte ratio; OS=overall survival; PFS=progression-free survival

Table A3. Univariate Cox proportional hazards model for progression-free survival

		***N* (events)**	**HR**	**95% CI**	***P*-value**
Gender	Male	131 (112)	1		0.1314
	Female	81 (61)	0.79	0.58-1.08	
ECOG PS	0-1	150 (123)	1		< 0.0001
	2-3	62 (50)	2.06	1.48-2.88	
Primary tumour location	Head/Head and body	122 (95)	1		0.0024
	Other	90 (78)	1.6	1.18-2.16	
Age	< 65 years	90 (74)	1		0.0465
	≥ 65 years	113 (99)	1.36	1.00-1.85	
Stage	IV	45 (35)	1		0.0183
	I-III	158 (138)	0.63	0.43-0.93	
Differentiation grade	Poor and moderate	110 (95)	1		0.0072
	Well	64 (50)	0.62	0.44-0.88	
Number of metastatic sites	1	131 (107)	1		0.1152
	2	58 (49)	1.16	0.82-1.62	
	≥ 3	21 (16)	1.74	1.02-2.96	
Liver metastases	Yes	132 (116)	1		0.2177
	No	71 (57)	0.82	0.60-1.13	
Resection of primary tumour	No	153 (129)	1		0.0127
	Yes	59 (44)	0.64	0.45-0.91	
Adjuvant chemotherapy	No	163 (141)	1		0.3235
	Yes	38 (31)	0.82	0.56-1.21	
Albumin (g/l)	< 40	132 (111)	1		0.0012
	≥ 40	69 (53)	0.58	0.42-0.81	
CEA (ng/ml)	< 8	115 (96)	1		0.0585
	≥ 8	80 (64)	1.36	0.99-1.87	
CA19-9 (UI/ml)	< 1000	120 (94)	1		0.0073

	≥ 1000	77 (67)	1.55	1.12-2.12	
NLR at baseline	≤ 5	162 (130)	1		< 0.0001
	> 5	50 (43)	2.38	1.67-3.38	

Abbreviations: ECOG PS=Eastern Cooperative Oncology Group Performance Status; CA 19-9=carbohydrate antigen; CEA=carcinoembryonic antigen; NLR=neutrophil-to-lymphocyte ratio

Table A4. Multivariate Cox proportional hazards model for progression-free survival

		***N* (events)**	**HR**	**95% CI**	***P*-value**
		188 (153)			
NLR at baseline	≤ 5		1		0.0026
	> 5		1.80	1.23-2.65	
Primary tumour location	Head/Head and body		1		0.0019
	Other		1.70	1.22-2.37	
Albumin (g/l)	< 40		1		0.0003
	≥ 40		0.52	0.37-0.74	
CA19-9 (UI/ml)	< 1000		1		0.0205
	≥ 1000		1.49	1.06-2.08	

Abbreviations: CA 19-9=carbohydrate antigen; NLR=neutrophil-to-lymphocyte ratio

Table A5. Comparison of characteristics between patients with and without NLR data at baseline in the AFUGEM cohort

Characteristics	Total	no NLR data at baseline	NLR information at baseline	*P*-value
	***N* = 114** ***n* (%)**	***N* = 29** ***n* (%)**	***N* = 85** ***n* (%)**	
Age*				0.3488
Median	66.1	64.4	66.4	
IQR	61.5-72.7	62.9-70.2	61.3-73.1	
Min-max	45.0-85.7	45.0-85.0	46.6-85.7	
Missing	0	0	0	
Gender				0.7215
Male	70 (61.4)	17 (58.6)	53 (62.3)	
Female	44 (38.6)	12 (41.4)	32 (37.7)	
ECOG PS				0.0075
0	37 (32.5)	6 (20.7)	31 (36.5)	
1	59 (51.8)	22 (75.9)	37 (43.5)	
2	18 (15.8)	1 (3.5)	17 (20.0)	
3	0	0	0	
Tumour location				0.2311
Head	43 (38.1)	6 (21.4)	37 (43.5)	
Body	25 (22.1)	7 (25.0)	18 (21.2)	
Tail	24 (21.2)	7 (25.0)	17 (20.0)	
Head and body	7 (6.2)	2 (7.1)	5 (5.9)	
Body and tail	14 (12.4)	6 (21.4)	8 (9.4)	
Missing	1	1	0	

Stage				1
I/II	5 (4.4)	1 (3.5)	4 (4.7)	
III	2 (1.8)	0	2 (2.4)	
IV	107 (93.9)	28 (96.6)	79 (92.9)	
Differentiation grade				0.2433
Well	23 (31.5)	2 (13.3)	21 (36.2)	
Moderate	41 (56.2)	11 (73.3)	30 (51.7)	
Poor	9 (12.3)	2 (13.3)	7 (12.1)	
Missing	41	14	27	
Metastatic site				0.7603
1	72 (63.2)	19 (65.5)	53 (62.4)	
≥ 2	42 (36.9)	10 (34.5)	32 (37.7)	
Liver metastases	86 (75.4)	21 (72.4)	65 (76.5)	0.6612
Resection of primary tumour	15 (13.2)	1 (3.5)	14 (16.5)	0.0615
Adjuvant chemotherapy				1
Yes	5 (4.5)	1 (3.6)	4 (4.8)	
Missing	3	1	2	
Albumin (g/l)				0.1626
Median	38.3	36	39.3	
IQR	34-42	33.2-40	34.7-42	
CA 19-9 (UI/ml)				0.3262
Median	891	1375.5	812.4	
IQR	65-9205	186.3-9928.5	50.2-9205	
CEA (ng/ml)				0.454
Median	6	9.96	5.5	
IQR	2.5-23	2.7-47.4	2.5-19.6	
Treatment arm				0.3839
Gemcitabine plus nab-paclitaxel	39 (34.2)	8 (27.6)	31 (36.5)	

Simplified leucovorin and fluorouracil plus nab-paclitaxel	75 (65.8)	21 (72.4)	54 (63.5)	
Death	79 (69.30)	21 (72.41)	58 (68.24)	0.6736
Progression	62 (54.4)	18 (62.1)	44 (51.8)	0.336
OS in months				
Median 95% CI	10.02 (8.8-13.6)	9.49 (5.9-16.4)	10.81 (8.8-14.1)	0.7587
PFS in months				
Median 95% CI	6.41 (4.8-7.7)	4.86 (1.9-8)	7.20 (4.9-8.1)	0.2727

Abbreviations: IQR= interquartile range; ECOG PS=Eastern Cooperative Oncology Group Performance Status; CA 19-9=carbohydrate antigen; CEA=carcinoembryonic antigen; NLR=neutrophil-to-lymphocyte ratio; OS=overall survival; PFS=progression-free survival

Table A6. Comparison of characteristics between patients with and without NLR data at baseline in the GHPS cohort

Characteristics	Total	no NLR information at baseline	NLR information at baseline	*P*-value
	***N* = 145** ***n* %**	***N* = 18** ***n* %**	***N* = 127** ***n* %**	
Age*				0.2957
Median	65.4	67.2	65.3	
IQR	60.4-72.9	64.5-73.2	60.2-72.9	
Gender				0.3548
Male	87 (60.0)	9 (50.0)	78 (61.4)	
Female	58 (40.0)	9 (50.0)	49 (38.6)	
ECOG PS				0.4857
0	41 (28.3)	5 (27.8)	36 (28.4)	
1	50 (34.5)	4 (22.2)	46 (36.2)	
2	41 (28.3)	6 (33.3)	35 (27.6)	
3	13 (9.0)	3 (16.7)	10 (7.9)	
Tumour location				0.2591
Head	80 (55.2)	10 (55.6)	70 (55.1)	
Body	22 (15.2)	2 (11.1)	20 (15.8)	
Tail	29 (20.0)	2 (11.1)	27 (21.2)	
Head and body	14 (9.7)	4 (22.2)	10 (7.3)	
Body and tail	0	0	0	
Stage				0.2532
I/II	38 (26.2)	2 (11.1)	36 (28.4)	
III	5 (3.5)	0	5 (3.9)	
IV	102 (70.3)	16 (88.9)	86 (67.7)	
Differentiation				0.7864

grade				
Well	51 (38.1)	8 (44.4)	43 (37.1)	
Moderate	62 (46.3)	7 (38.9)	55 (47.4)	
Poor	21 (15.7)	3 (16.7)	18 (15.5)	
Metastatic site				0.0192
1	84 (58.7)	6 (33.3)	78 (62.4)	
≥ 2	59 (41.3)	12 (66.7)	47 (37.6)	
Liver metastases	76 (52.4)	3 (16.7)	73 (57.5)	0.0012
Resection of primary tumour	49 (33.8)	4 (22.2)	45 (35.4)	0.2674
				0.3999
Yes	39 (26.9)	3 (16.7)	36 (28.4)	
Albumin (g/l)				0.0414
Median	36	31.5	36.5	
IQR	30-39	27-37	31-40	
CA 19-9 (UI/ml)				0.9327
Median	355	213.5	369	
IQR	29-2555.5	10-18765	31.5-2092	
CEA (ng/ml)				0.2671
Median	4.5	6.5	4	
IQR	2-18	3-43.5	2-17	
Death	1103 (71.03)	10 (55.6)	93 (73.2)	0.1219
Progression	83 (57.2)	7 (38.9)	76 (59.9)	0.0926
OS				
Median 95%CI	10.32 (8.4-13.8)	11.70 (2.9-24.2)	10.32 (8.3-13.8)	0.9578
PFS				
Median 95%CI	4.57 (3.3-5.6)	3.29 (2.4-19.3)	4.60 (3.3-5.6)	0.5935

Abbreviations: IQR= interquartile range; ECOG PS=Eastern Cooperative Oncology Group Performance Status; CA 19-9=carbohydrate antigen; CEA=carcinoembryonic antigen; NLR=neutrophil-to-lymphocyte ratio; OS=overall survival; PFS=progression-free survival

Table A7. Comparison of patient characteristics according to NLR at baseline in the AFUGEM cohort

Characteristics		**NLR data at baseline**	**NLR ≤ 5**	**NLR > 5**	***P*-value**
		***N* = 85** ***n* %**	***N* = 64** ***n* %**	***N* = 21** ***n* %**	
Age*					0.3949
	Median	66.3	66.1	68.6	
	IQR	61.3-73.1	59.7-73.0	62.54-73.11	
Gender*					0.2771
	Male	53 (62.3)	42 (65.6)	11 (52.9)	
	Female	32 (37.6)	22 (34.9)	1 0(47.6)	
ECOG PS*					0.0503
	0	31 (36.5)	26 (40.6)	5 (23.8)	
	1	37 (43.5)	29 (45.3)	8 (38.1)	
	2	17 (20.0)	9 (14.1)	8 (38.1)	
	3	0	0	0	
Tumour location					0.511
	Head	37 (43.5)	29 (45.3)	8 (38.1)	
	Body	18 (21.2)	11 (17.2)	7 (33.3)	
	Tail	17 (20.0)	13 (20.3)	4 (19.0)	
	Head and body	5 (5.9)	5 (7.8)	0	
	Body and tail	8 (9.4)	6 (9.9)	2 (9.5)	
	Missing	0	0	0	
Stage*					0.7559
	I/II	4 (4.7)	4 (6.2)	0	
	III	2 (2.3)	2 (3.1)	0	
	IV	79 (92.9)	58 (90.6)	21 (100)	
Differentiation grade					0.0834

	Well	21 (36.2)	15 (32.6)	6 (50.0)	
	Moderate	30 (51.7)	27 (58.7)	3 (25.0)	
	Poor	7 (12.1)	4 (8.7)	3 (25.0)	
	Missing	27	18	9	
Metastatic site					0.6382
	1	53 (62.3)	39 (60.9)	14 (66.7)	
	≥ 2	32 (37.6)	25 (39.1)	7 (33.3)	
	Missing	0	0	0	
Liver metastases*		65 (76.5)	50 (78.1)	15 (71.4)	0.5605
Resection of primary tumour*		14 (16.5)	13 (20.3)	1 (4.8)	0.1721
Adjuvant chemotherapy					0.5678
	Yes	4 (4.8)	4 (6.4)	0	
	Missing	2	2	0	
Albumin (g/L)*					0.1735
	Median	39.3	40	37	
	IQR	34.68-42	34.95-42.05	31.72-40	
CA 19-9 (UI/ml)					0.263
	Median	812.4	561.7	2320.5	
	IQR	50.20-9205	43-7477	53.35-10453	
	Missing	8	5	3	
CEA (ng/ml)					0.2627
	Median	5.5	5.36	8.6	
	IQR	2.5-19.6	2.5-19.10	3-112.2	
	Missing	10	6	4	
Treatment*					0.7307
	Gemcitabine plus nab-paclitaxel	31 (36.5)	24 (37.5)	7 (33.3)	

	Simplified leucovorin and fluorouracil plus nab-paclitaxel	54 (63.5)	40 (62.5)	14 (66.7)	
Death		58 (68.2)	38 (69.4)	20 (95.2)	0.0022
Progression		44 (51.7)	30 (46.9)	14 (66.7)	0.1153
OS in months					
	Median 95%CI	10.81 (8.77-14.09)	13.77 (9.5-17.6)	4.63 (2.3-6.34)	< 0.0001
PFS in months					
	Median 95%CI	7.20 (4.93-8.12)	8.21 (7.2-10.3)	2.33 (1.6-4.0)	< 0.0001

*No missing data

Abbreviations: IQR= interquartile range; ECOG PS=Eastern Cooperative Oncology Group Performance Status; CA 19-9=carbohydrate antigen; CEA=carcinoembryonic antigen; NLR=neutrophil-to-lymphocyte ratio; OS=overall survival; PFS=progression-free survival

Table A8. Comparison of patient characteristics according to NLR at baseline in the GHPS cohort

Characteristics		**NLR information at baseline**	**NLR ≤ 5**	**NLR > 5**	***P*-value**
		***N* = 127** ***n* (%)**	***N* = 98** ***n* (%)**	***N* = 29** ***n* (%)**	
Age					0.0953
	Median	65.25	64.8	67.79	
	IQR	60.15-72.88	58.88-72.22	61.98-76.52	
	Missing	9	4	5	
Gender*					0.7247
	Male	78 (61.4)	61 (62.2)	17(58.6)	
	Female	49 (38.6)	37 (37.8)	12 (41.4)	
ECOG PS*					0.0014
	0	36 (28.3)	33 (33.7)	3 (10.3)	
	1	46 (36.2)	38 (38.8)	8 (27.6)	
	2	35 (27.6)	23 (23.5)	12 (41.4)	
	3	10 (7.9)	4 (4.1)	6 (20.7)	
Tumour location*					0.2148
	Head	70 (55.1)	57 (58.2)	13 (44.8)	
	Body	20 (15.7)	15 (15.3)	5 (17.2)	
	Tail	27 (21.3)	17 (17.3)	10 (34.5)	
	Head and body	10 (7.9)	9 (9.2)	1 (3.4)	
	Body and tail	0	0	0	
Stage*					0.0848
	I/II	36 (28.35)	32 (32.65)	4 (13.79)	
	III	5 (3.94)	3 (3.06)	2 (6.90)	

	IV	86 (67.72)	63 (64.29)	23 (79.31)	
Differentiation grade					0.3723
	Well	43 (37.1)	37 (40.2)	6 (25.0)	
	Moderate	55 (47.4)	41 (44.6)	14 (58.3)	
	Poor	18 (15.5)	14 (15.2)	4 (16.7)	
	Missing	11	6	5	
Metastatic site					0.0258
	1	78 (62.40)	65 (67.71)	13 (44.83)	
	≥ 2	47 (37.60)	31 (32.29)	16 (55.17)	
	Missing	2	2	0	
Liver metastases*		73 (57.5)	53 (54.1)	20 (69.0)	0.1544
Resection of primary tumour*		45 (35.4)	39 (39.8)	6 (20.7)	0.0588
Adjuvant chemotherapy		36 (28.3)	31 (31.6)	5 (17.2)	0.1309
Albumin (g/L)					0.0574
	Median	36.5	37	32	
	IQR	31-40	33-40	27-39	
	Missing	11	8	3	
CA 19-9 (UI/ml)					0.2087
	Median	369	341	1672	
	IQR	31.5-2092	36-1200	14-10000	
	Missing	7	5	2	
CEA (ng/ml)					0.0922
	Median	4	4	9	
	IQR	2-17	2-15	3-29	
	Missing	7	5	2	
Death		93 (73.2)	71 (72.4)	22 (75.9)	0.7154

Progression		76 (59.8)	68 (69.4)	8 (27.6)	< 0.0001
OS in months					
	Median 95% CI	10.32 (8.4-13.8)	13.47 (10.1-18.3)	2.53 (1.2-5.2)	< 0.0001
PFS in months					
	Median 95% CI	4.57 (3.3-5.6)	5.36 (4.07-7.39)	1.61 (1.18-3.91)	0.0124

*No missing data

Abbreviations: IQR= interquartile range; ECOG PS=Eastern Cooperative Oncology Group Performance Status; CA 19-9=carbohydrate antigen; CEA=carcinoembryonic antigen; NLR=neutrophil-to-lymphocyte ratio; OS=overall survival; PFS=progression-free survival

Table A9. Chemotherapy regimen in GHPS cohort (*n*=145)

Chemotherapy	Frequency	Percent (%)	Cumulative frequency	Cumulative percentage (%)
None	9	6.4	9	6.2
Gemcitabine	29	20.7	38	26.2
Gemcitabine-Oxaliplatin	2	1.4	40	27.6
FOLFIRINOX	47	33.6	87	60.0
FOLFOX	30	21.4	117	80.7
FOLFIRI	6	4.3	123	84.8
Gemcitabine-Abraxane	2	1.4	125	86.2
5-FU-Abraxane	5	3.6	130	89.7
Gemcitabine-Erlotinib	8	5.7	138	95.2
Erlotinib	1	0.7	139	95.9
Maestro Trial	1	0.7	140	96.6
Missing	5	3.6	145	100

Table A10. Dosages of different chemotherapy regimens

Chemotherapy regimens	**Dosage**
Gemcitabine monotherapy	• Gemcitabine: 1000 mg/m^2, days 1, 8, 15 One cycle every 4 weeks
Gemcitabine and nab-paclitaxel	• Gemcitabine: 1000 mg/m^2, days 1, 8, 15 • Nab-paclitaxel: 125 mg/m^2, days 1, 15, One cycle every 4 weeks
5-FU and nab-paclitaxel	• Nab-paclitaxel: 125 mg/m^2 • Leucovorin, 400 mg/m^2 and 5-FU, 400 mg/m^2 given as a bolus followed by 2400 mg/m^2 given as a 46-hour continuous infusion One cycle every 2 weeks
5-FU, irinotecan, and oxaliplatin	• Oxaliplatin, 85 mg/m^2 • Irinotecan, 180 mg/m^2 • Leucovorin, 400 mg/m^2 and 5-FU, 400 mg/m^2 given as a bolus followed by 2400 mg/m^2 given as a 46-hour continuous infusion One cycle every 2 weeks
5-FU and irinotecan	• Irinotecan, 180 mg/m^2 • Leucovorin, 400 mg/m^2 and 5-FU, 400 mg/m^2 given as a bolus followed by 2400 mg/m^2 given as a 46-hour continuous infusion One cycle every 2 weeks

5-FU and oxaliplatin	• Oxaliplatin, 85 mg/m^2 • Leucovorin, 400 mg/m^2 and 5-FU, 400 mg/m^2 given as a bolus followed by 2400 mg/m^2 given as a 46-hour continuous infusion One cycle every 2 weeks

Abbreviations: 5-FU = 5-fluorouracil

Table A11. Inclusion and exclusion criteria in the AFUGEM phase II trial

Inclusion criteria	1. Signed and dated informed consent, 2. Patients willing and able to comply with protocol requirements, 3. Histologically or cytologically proven adenocarcinoma of the pancreas, 4. Stage IV disease, 5. No prior therapy for metastatic disease (in case of previous adjuvant therapy, interval between the end of chemotherapy and relapse must be > 12 months), 6. At least one measurable or evaluable lesion as assessed by CT-scan or MRI according to RECIST v1.1, 7. Age ≥ 18 years, 8. ECOG PS 0 and 2, 9. Adequate hematologic function: neutrophils > 1.5 x 10^9/L; platelets > 100 x 10^9/L; haemoglobin≥9 g/dL, 10.Adequate renal function: serum creatinine level<150 µM, 11.Adequate liver function: AST (SGOT) and ALT (SGPT) ≤ 2.5 x ULN (≤ 5 x ULN

	in case of liver metastases), total bilirubin ≤ 1.5 x ULN, albumin ≥ 25 g/L, 12.Baseline evaluations performed before randomization: clinical and blood evaluations no more than 14 days prior to randomization, tumour assessment (CT-scan or MRI, evaluation of nonmeasurable lesions) no more than 21 days prior to randomization, 13.Female patients must be surgically sterile, or be postmenopausal, or must commit to using reliable and appropriate methods of contraception during the study and during at least 6 months after the end of study treatment (when applicable). All female patients with reproductive potential must have a negative pregnancy test (β HCG) within 72 h prior to starting nab-paclitaxel treatment. Breastfeeding is not allowed. Male patients must agree to use effective contraception in addition to having their partner use a contraceptive method as well during the trial and during at least 6 months after the end of the study treatment, 14.Registration with the French National Health Care System.
Exclusion criteria	1. Medical history or evidence of CNS metastasis upon physical examination, unless adequately treated (e.g., non-irradiated CNS metastasis, seizure not controlled with

	standard medical therapy), 2. Local or locally advanced disease (stage I to III), 3. Treatment with warfarin, 4. Uncontrolled hypercalcemia, 5. Pre-existing permanent neuropathy (NCI CTCAE grade ≥ 2), 6. Known dihydropyrimidine dehydrogenase deficiency, 7. Concomitant unplanned antitumor therapy (e.g., chemotherapy, molecular targeted therapy, immunotherapy), 8. Treatment with any other investigational medicinal product within 28 days prior to study entry, 9. Other serious and uncontrolled non-malignant disease (e.g., active infection requiring systemic therapy, coronary stenting or myocardial infarction, or stroke in the past 6 months), 10. HIV-infected patients or otherwise known to be HIV-positive with untreated hepatitis B or hepatitis C, 11. Medical history or active interstitial lung disease,

	12. Other concomitant or previous malignancy, except: i/ adequately treated in-situ carcinoma of the uterine cervix, ii/ basal or squamous cell carcinoma of the skin, iii/ cancer in complete remission for> 5 years, 13. Patients with known allergy to any excipient of study drugs, 14. Concomitant administration of prophylactic phenytoin and live attenuated virus vaccine such as yellow fever vaccine.

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Table A12. Evaluation of prognostic value of NLR evolution between baseline and Day 15, and between baseline and Day 30.

		n(events)	HR	95%CI	pvalue
OS	D15≤D0	106 (77)	1		0.3724
	D15>D0	65 (48)	0.85	0.59-1.22	
PFS	D15≤D0	106 (90)	1		0.9603
	D15>J0	65 (55)	1.01	0.72-1.41	

		n(events)	HR	95%CI	pvalue
OS	D30≤D0	97 (66)	1		0.6875
	D30>D0	60 (46)	1.08	0.74-1.58	
PFS	D30≤D0	97 (82)	1		0.9127
	D30>D0	60 (50)	0.98	0.69-1.40	

Figure A1. Overall survival in the both study cohorts

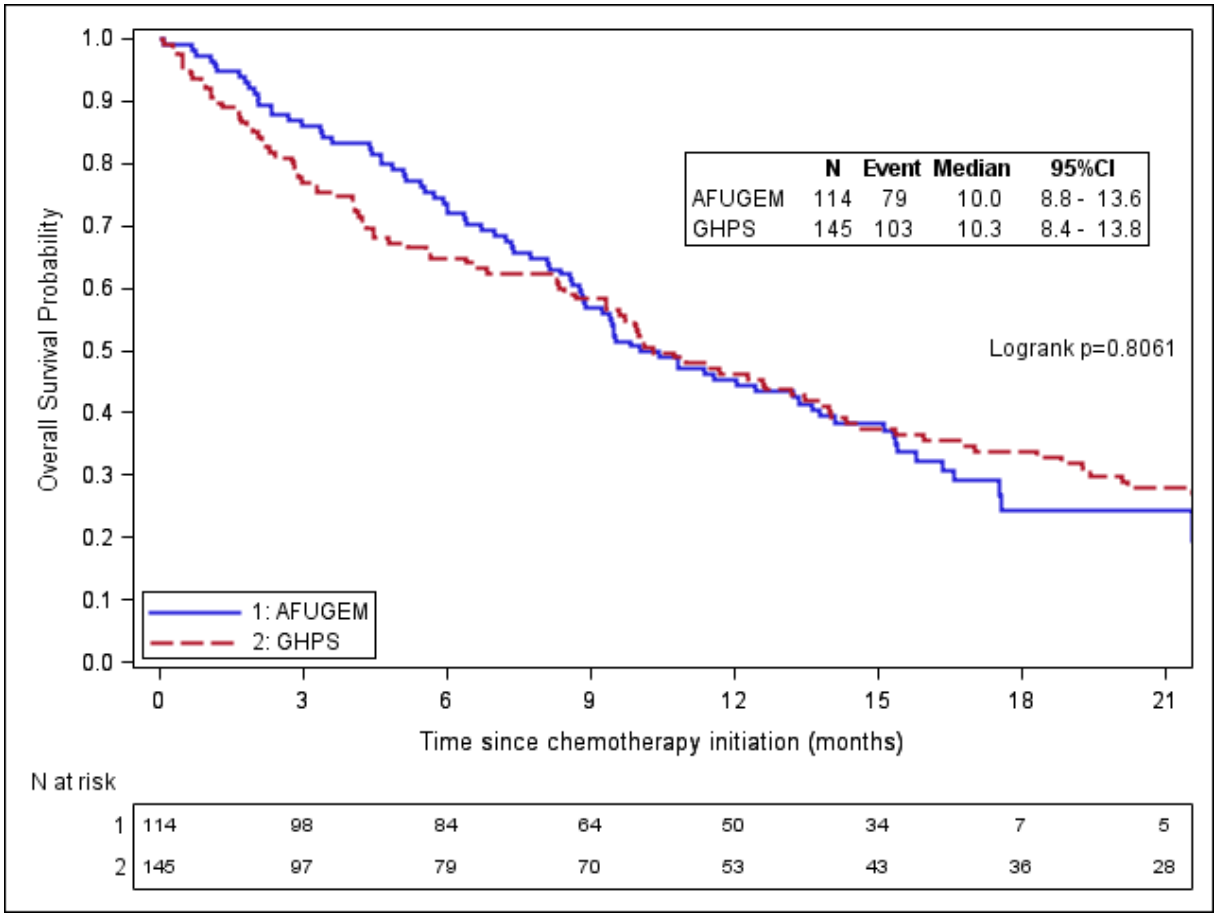


Figure A2. Progression-free survival in the both study cohorts

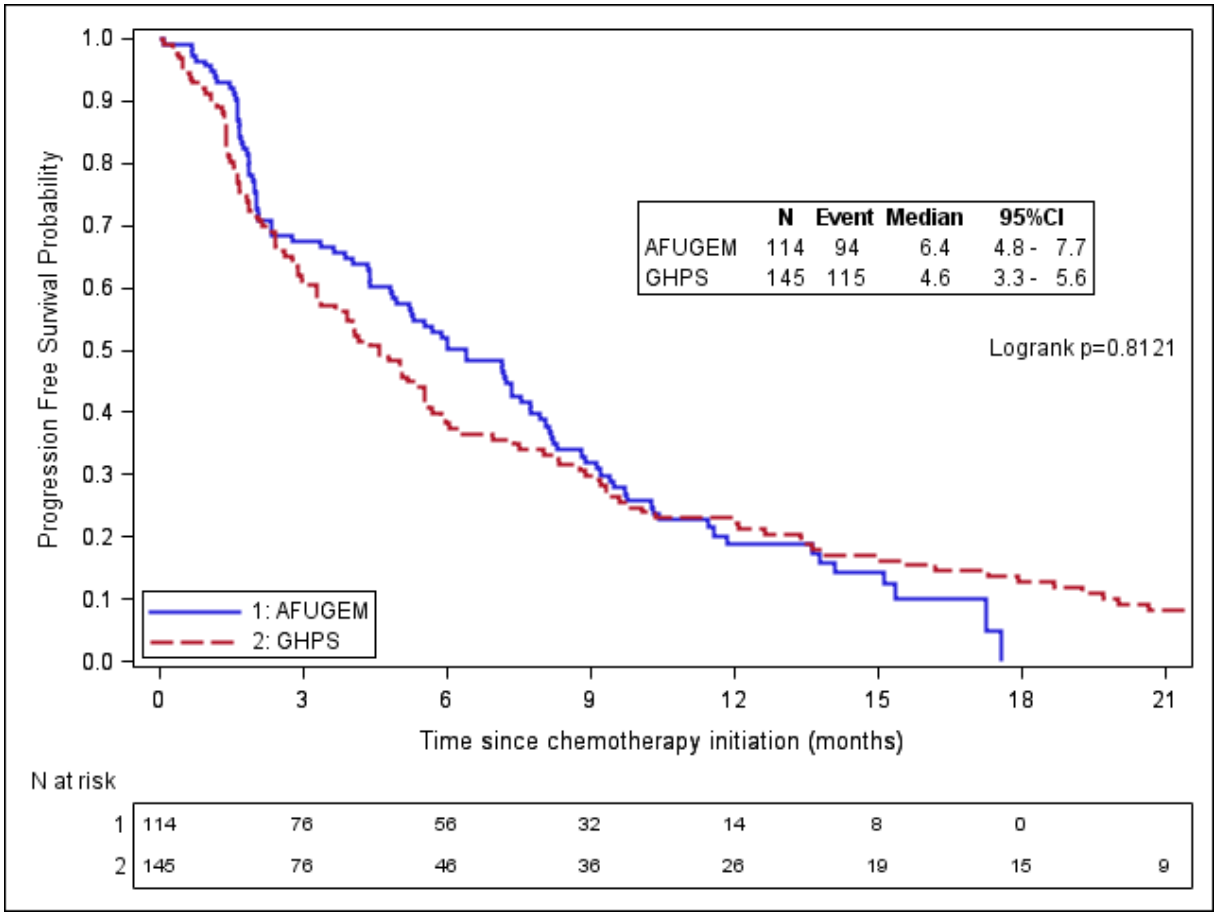


Figure A3. Overall survival according to availability of NLR at baseline

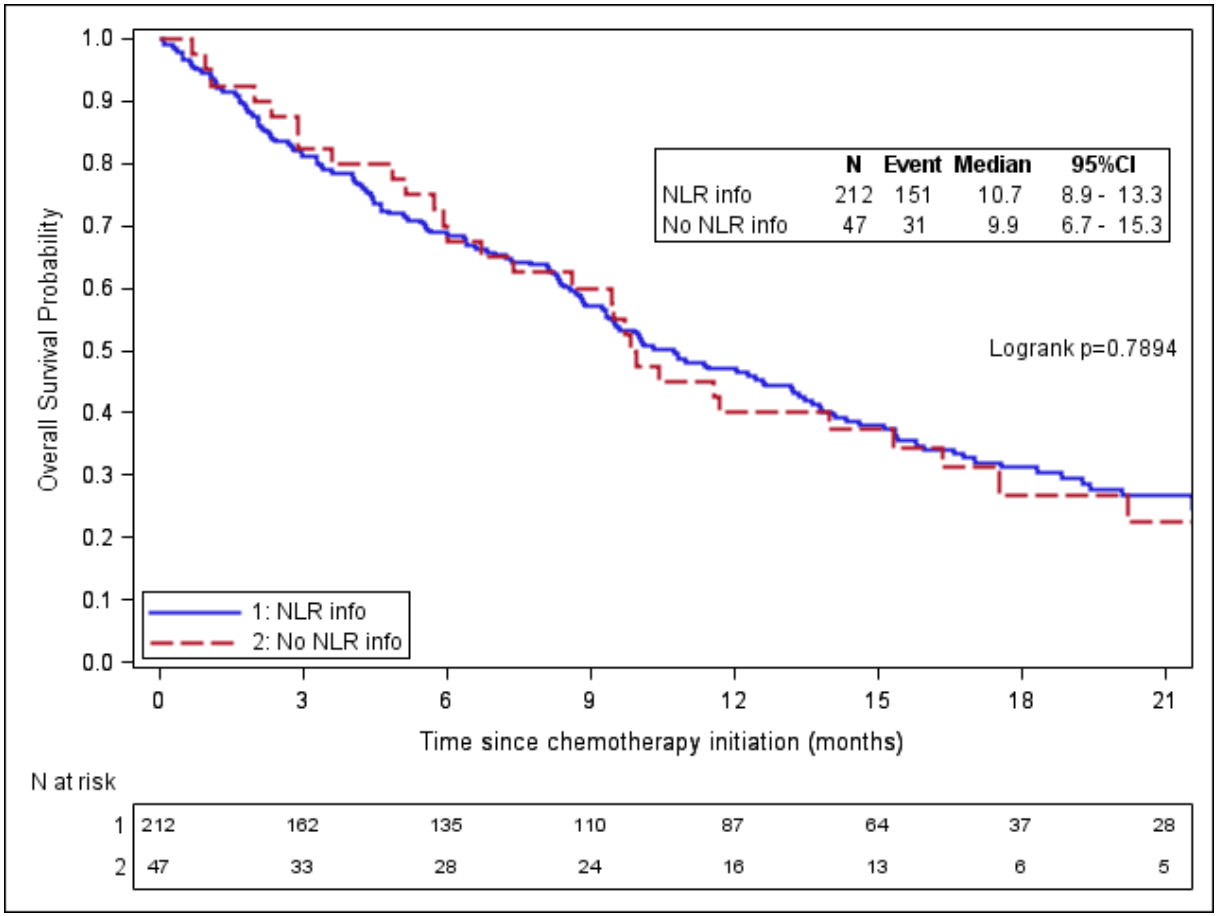


Figure A4. Progression-free survival according to availability of NLR at baseline

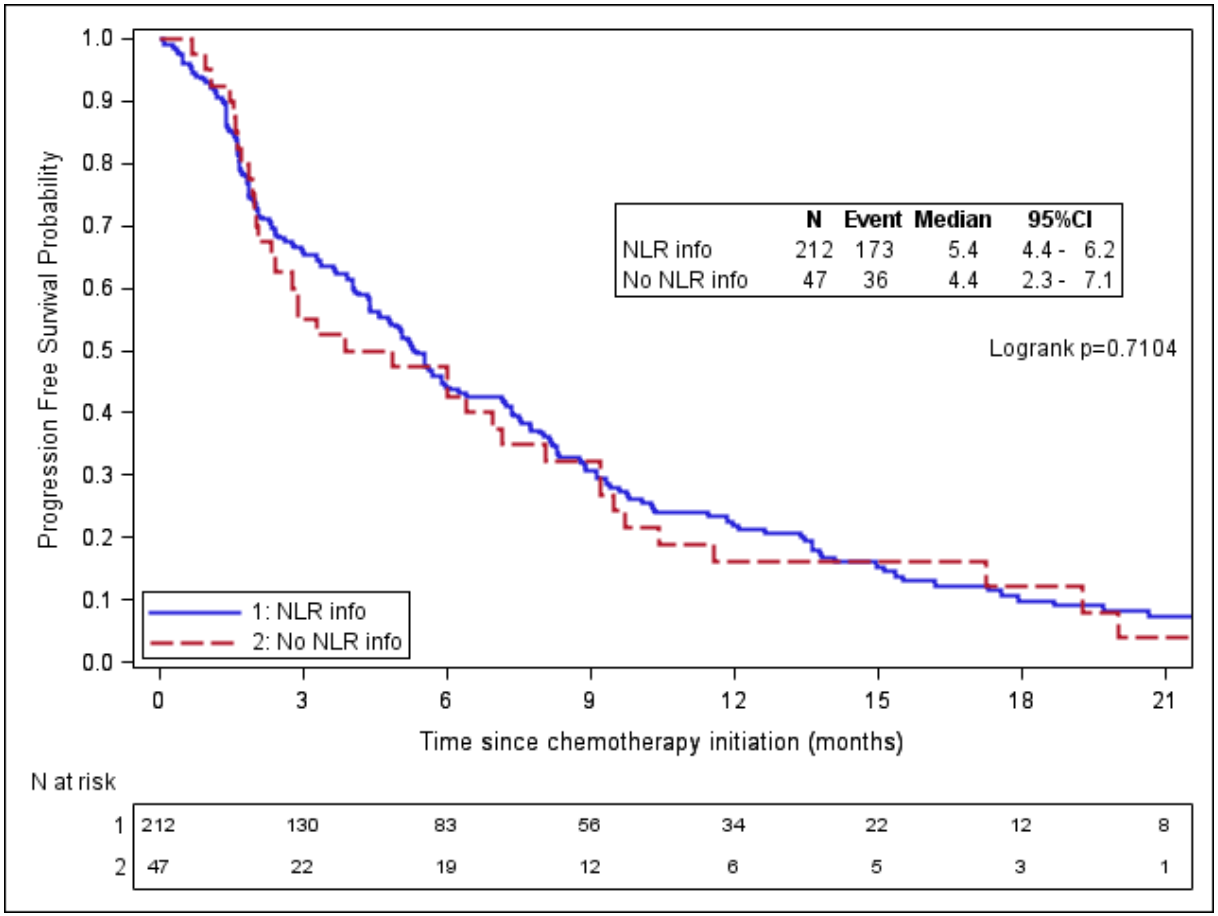


Figure A5. Relation between overall survival and NLR using a restricted cubic spline method

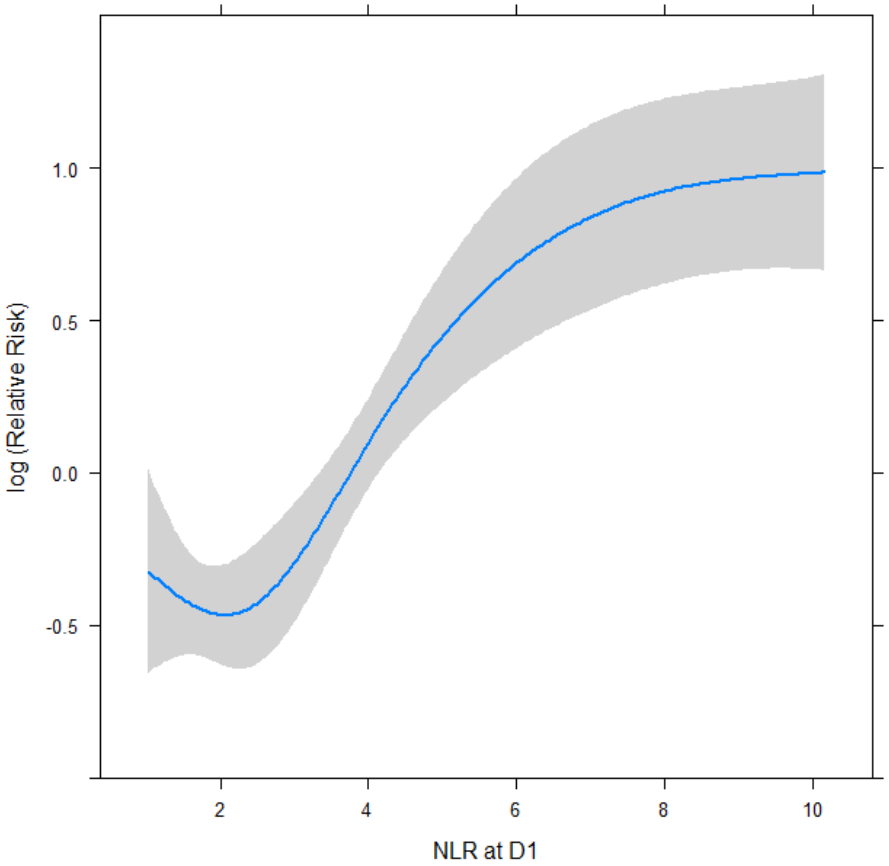
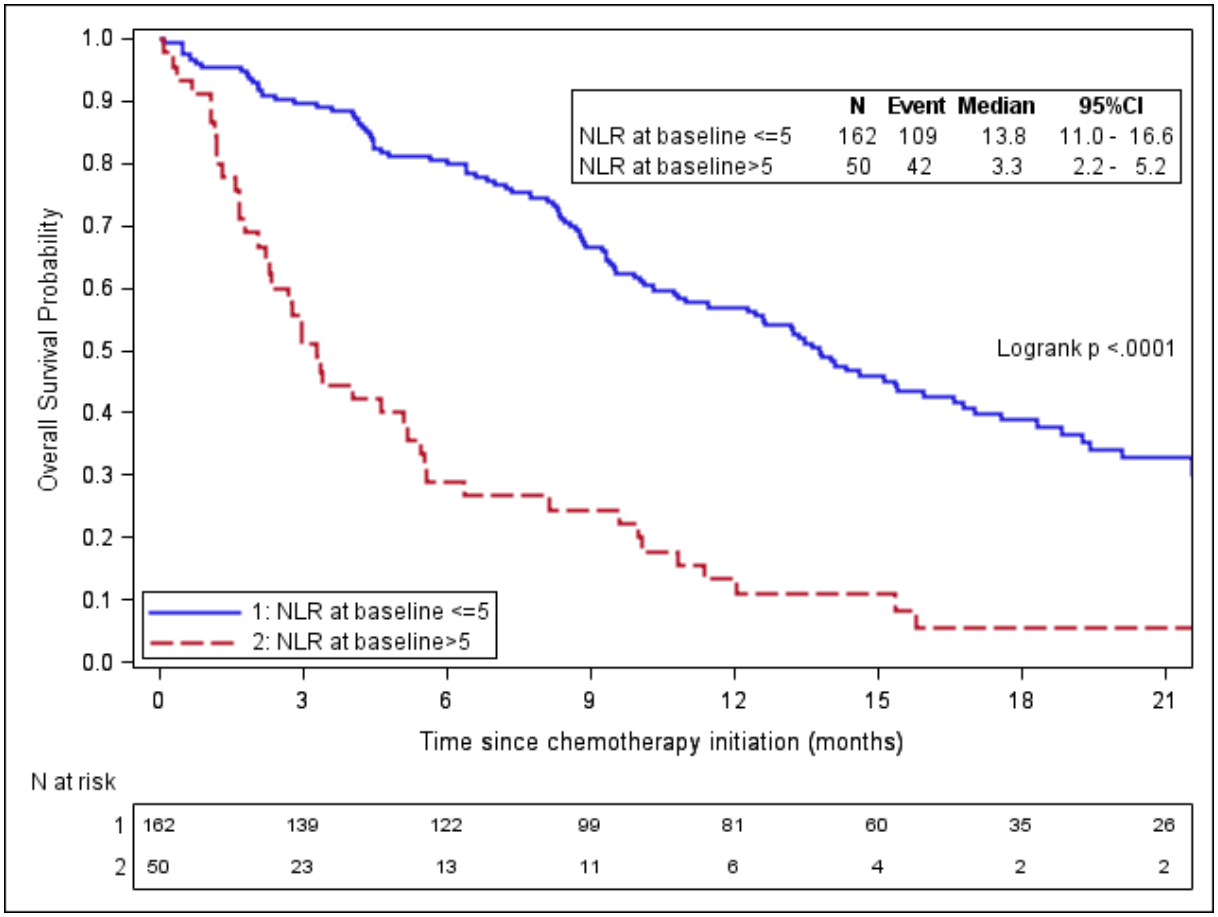
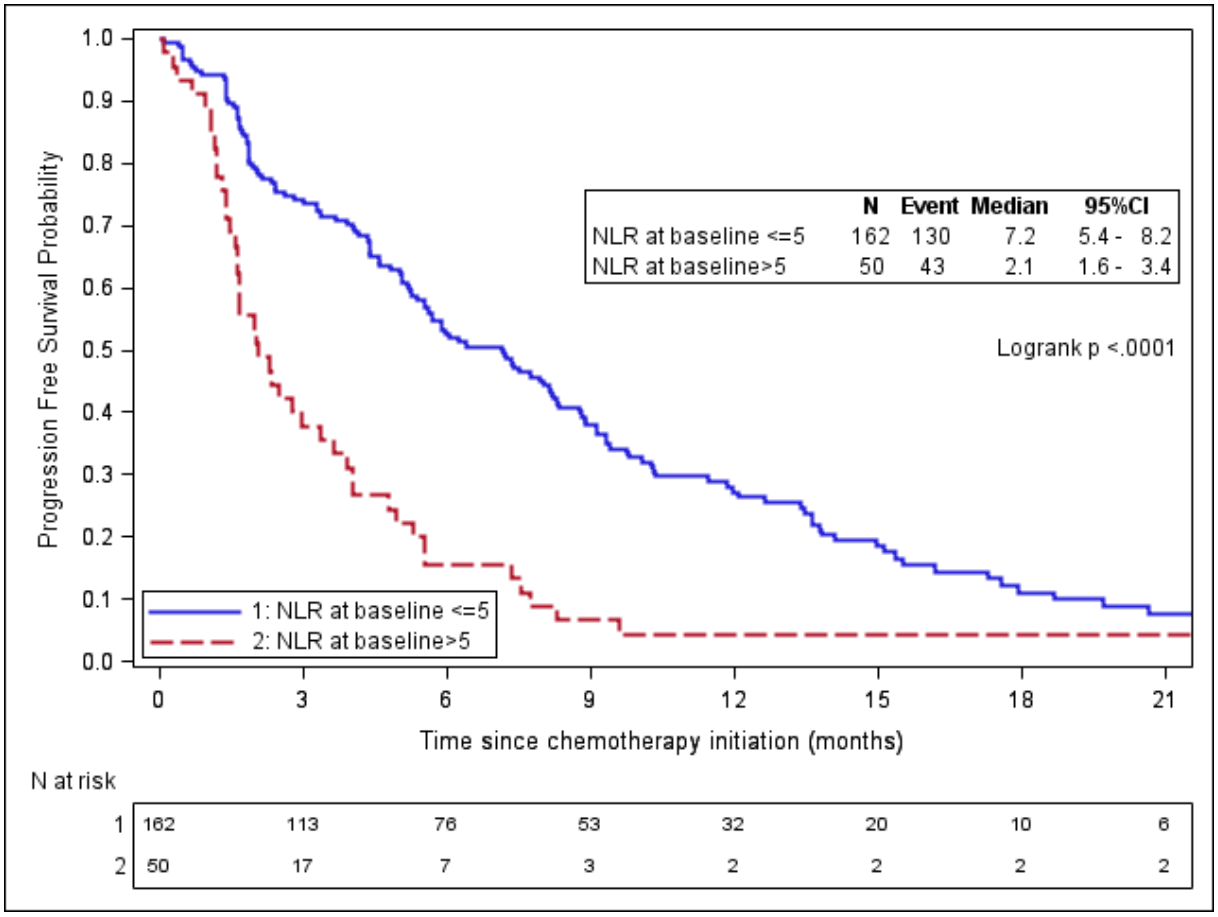


Figure A6. Overall survival according to a NLR baseline cut-off of 5



Reference: NLR ≤ 5=HR 3.22 (95% CI: 2.23-4.64); *P*-value < 0.0001

Figure A7. Progression-free survival according to a NLR baseline cut-off of 5



Reference: NLR ≤ 5=HR 2.37 (95% CI: 1.67-3.39); *P*-value < 0.0001

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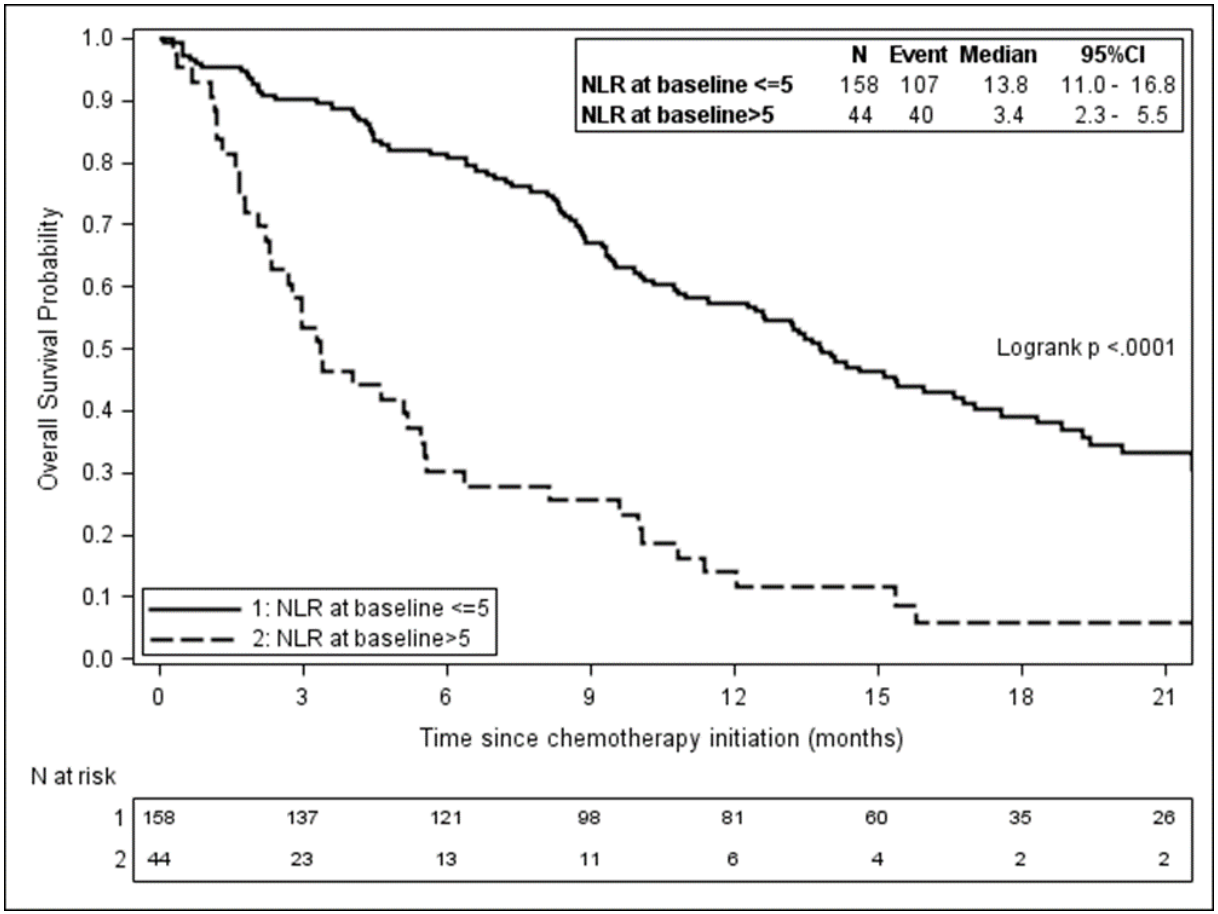
Figure A.8. Overall survival according to a NLR baseline cut-off of 5 (after exclusion of the 10 patients with ECOG PS 3)



HR=3.15 (95%CI: 2.17-4.58); p<0.0001

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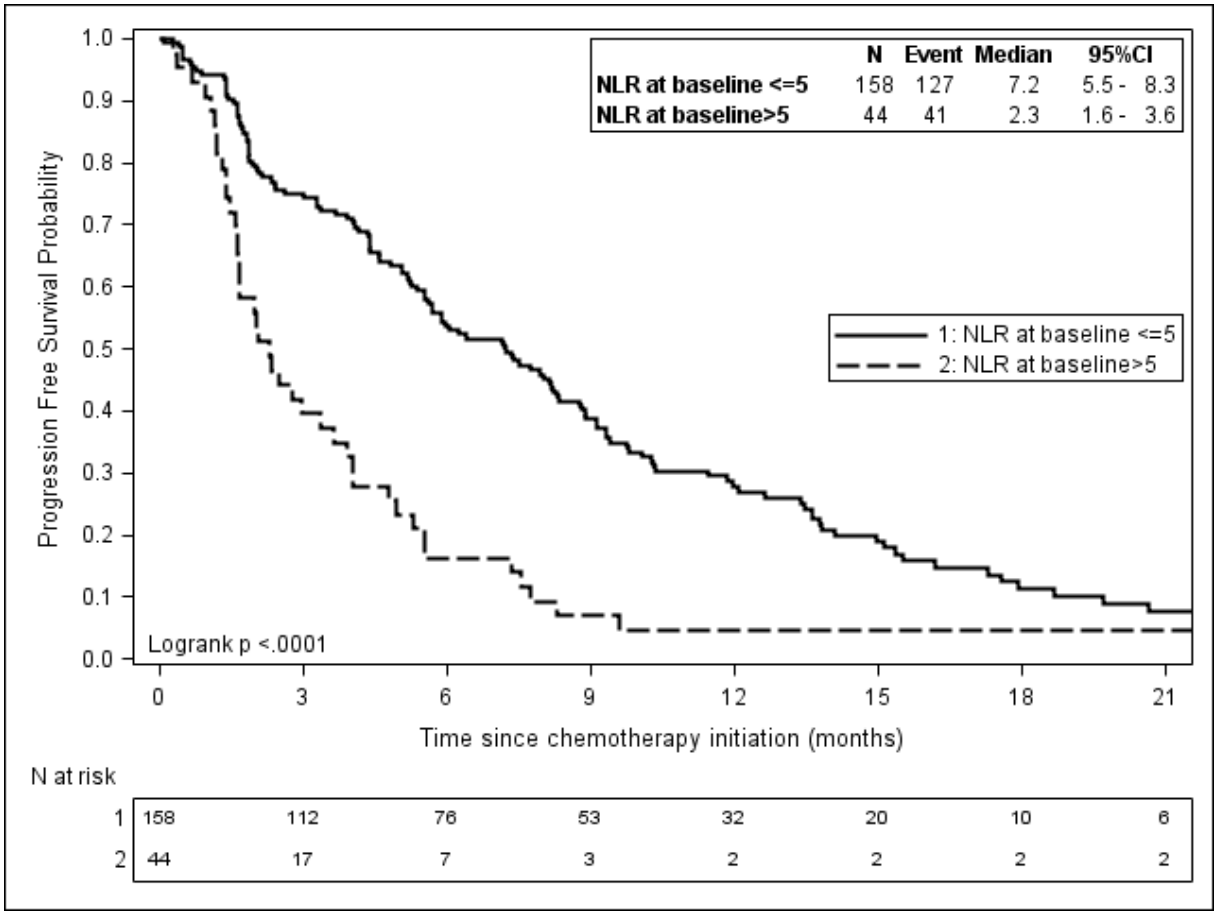
Figure A.9. Progression-free survival according to a NLR baseline cut-off of 5 (after exclusion of the 10 patients with ECOG PS 3)



HR=2.33 (95%CI: 1.62-3.34) ; p<0.0001

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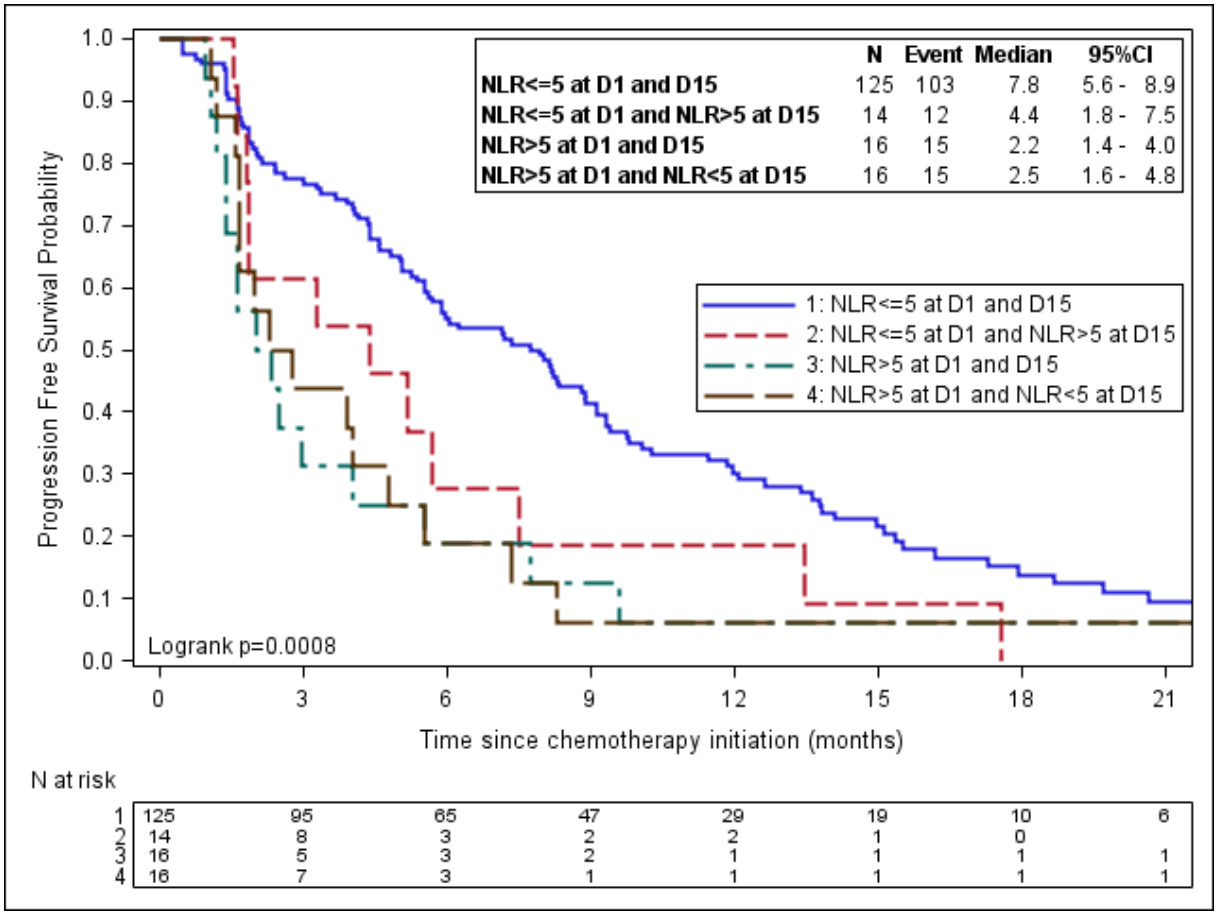
Figure A10. Progression-free survival according to NLR at baseline and NLR on day 15 of cycle



	HR	95% CI	*P*-value
NLR ≤5 at day 1and day 15	1	-	0.0012
NLR≤5 at day 1and NLR > 5 at day 15	1.72	0.94-3.13	0.0788

NLR>5 at day 1and day 15	2.34	1.35-4.04	0.0023
NLR>5 at day 1and NLR ≤ 5 at day15	2.12	1.23-3.68	0.0071

With a Cox time-varying covariate model and NLR transformed with log, HR=1.68 (95% CI 1.37-2.06); $P < 0.0001$.
With a Cox time-varying covariate model and NLR > 5 as factor, HR=2.26 (95% CI 1.57-3.25); $P < 0.0001$.

Figure A11. Overall survival according to NLR at baseline and NLR on day 15 of cycle (after exclusion of the 10 patients with ECOG PS 3)

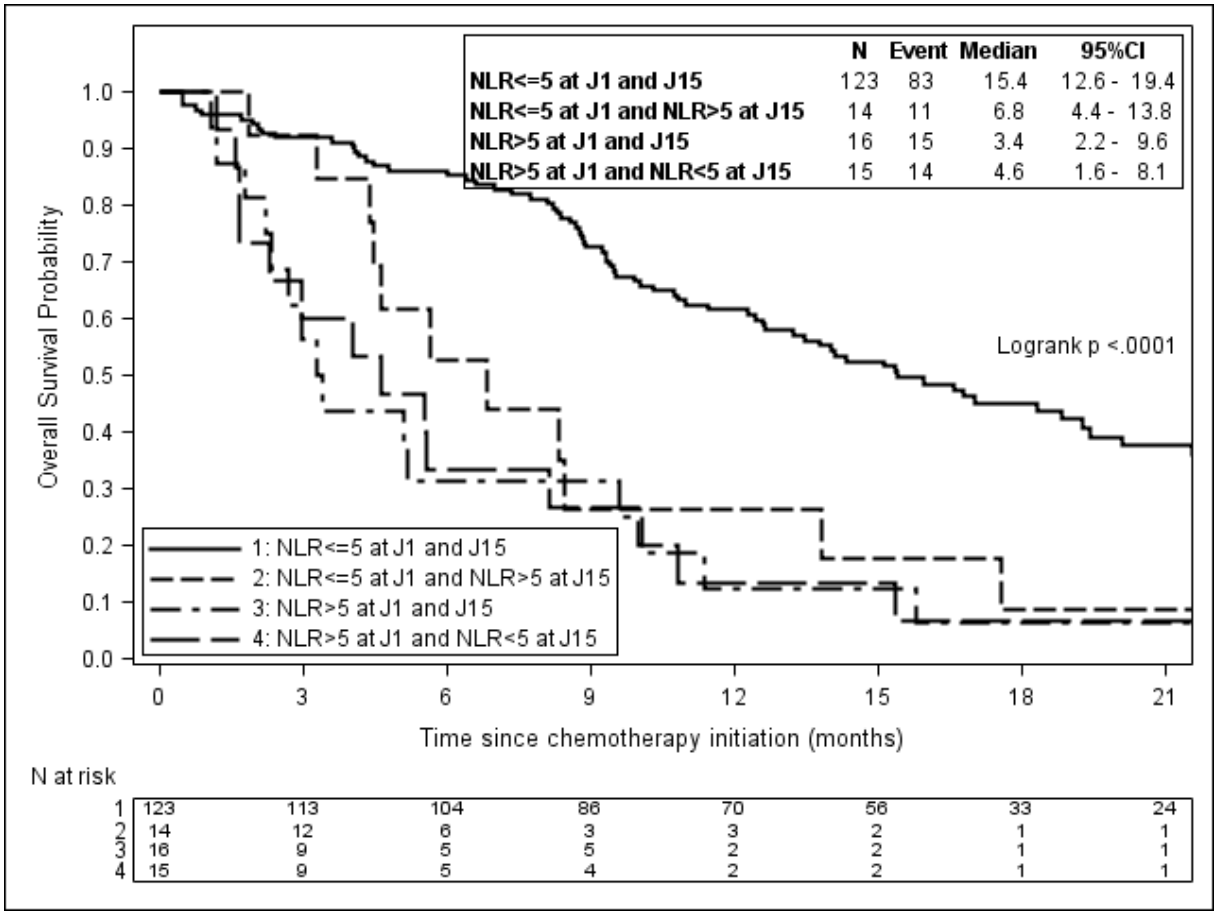


Figure A12. Progression-free survival according to NLR at baseline and NLR on day 15 of cycle (after exclusion of the 10 patients with ECOG PS 3)

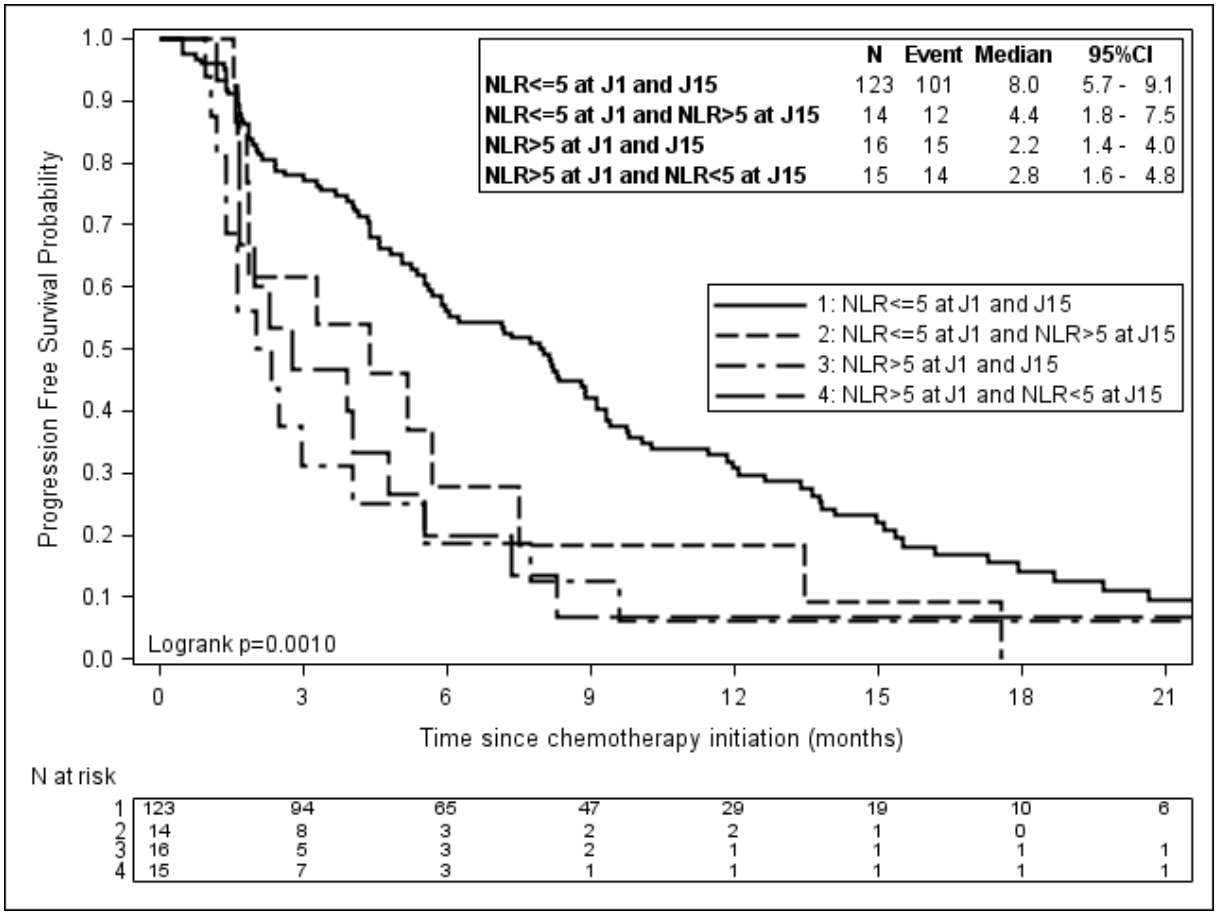
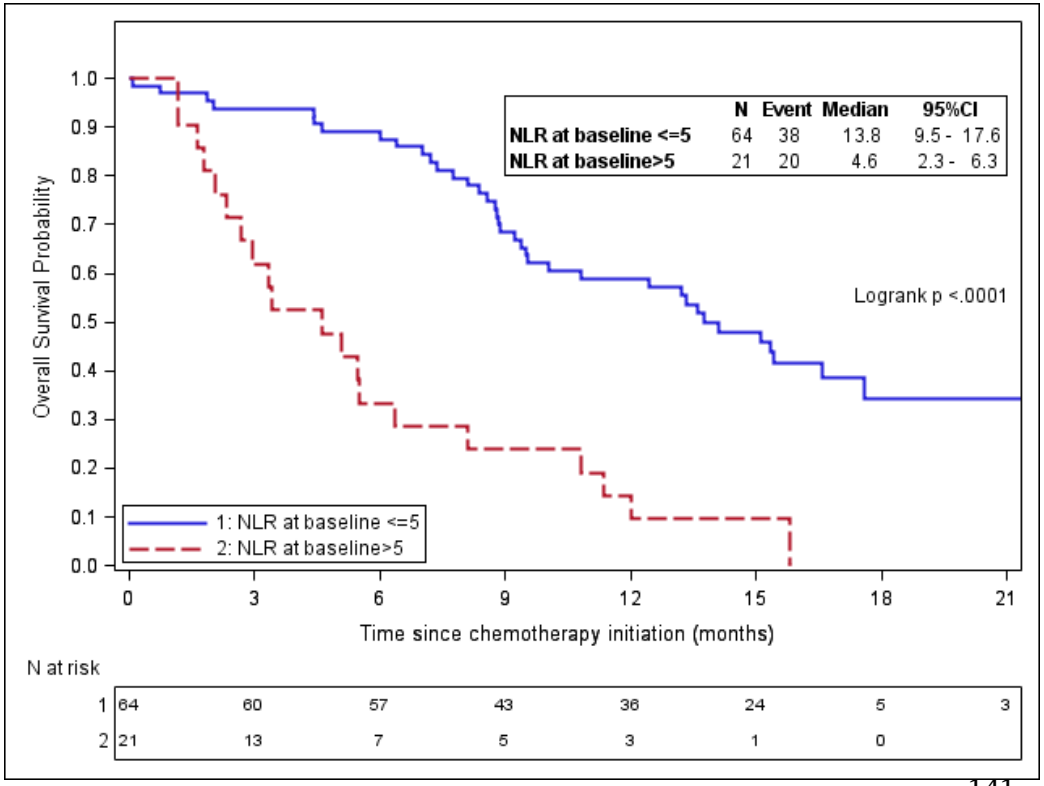
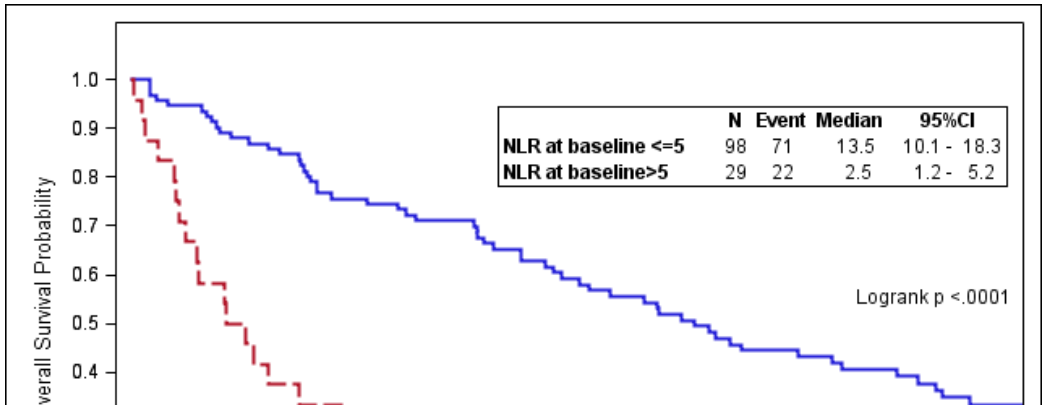


Figure A13. Overall survival according to NLR at baseline in A) the AFUGEM cohort and B) in the GHPS cohort

A



B



A

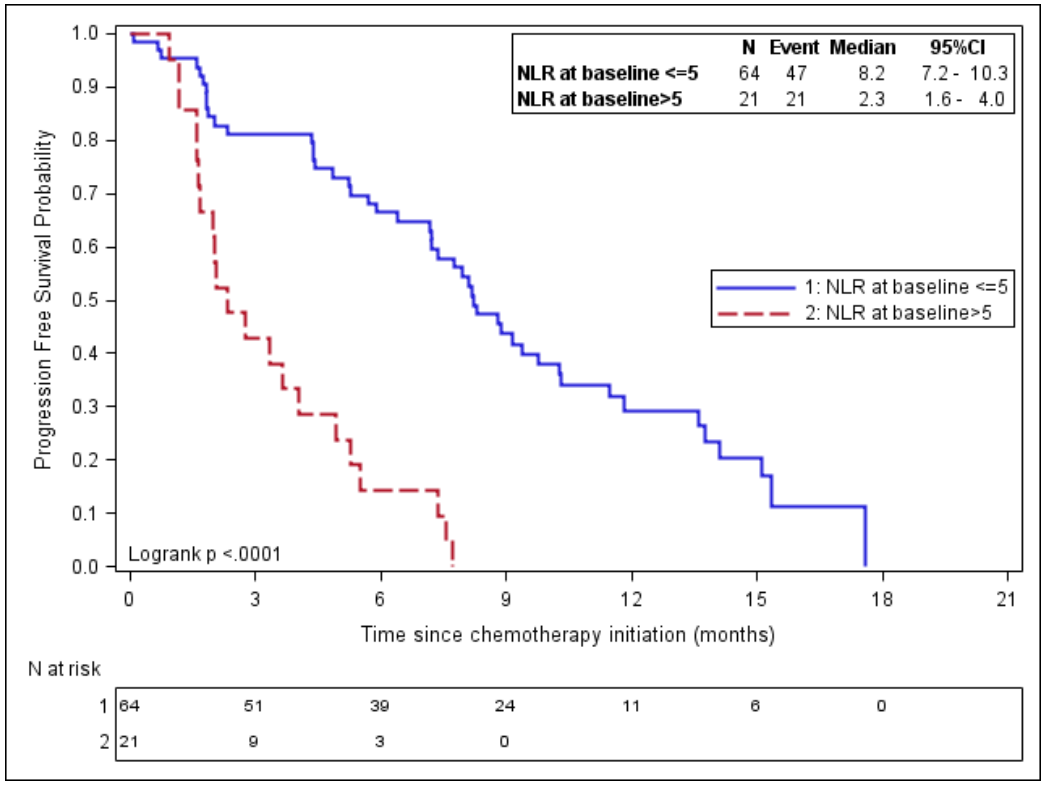
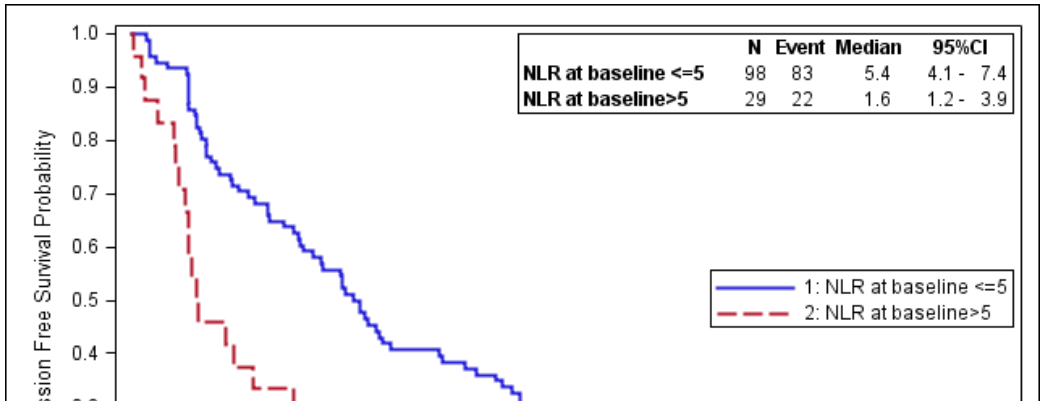


Figure A14. Progression-free survival according to NLR at baseline in A) the AFUGEM cohort and B) in the GHPS cohort

B


1.0
0.9
0.8
0.7
0.6
0.5
0.4
N Event Median 95%CI
NLR at baseline <=5 98 83 5.4 4.1 - 7.4
NLR at baseline>5 29 22 1.6 1.2 - 3.9
1: NLR at baseline <=5
2: NLR at baseline>5

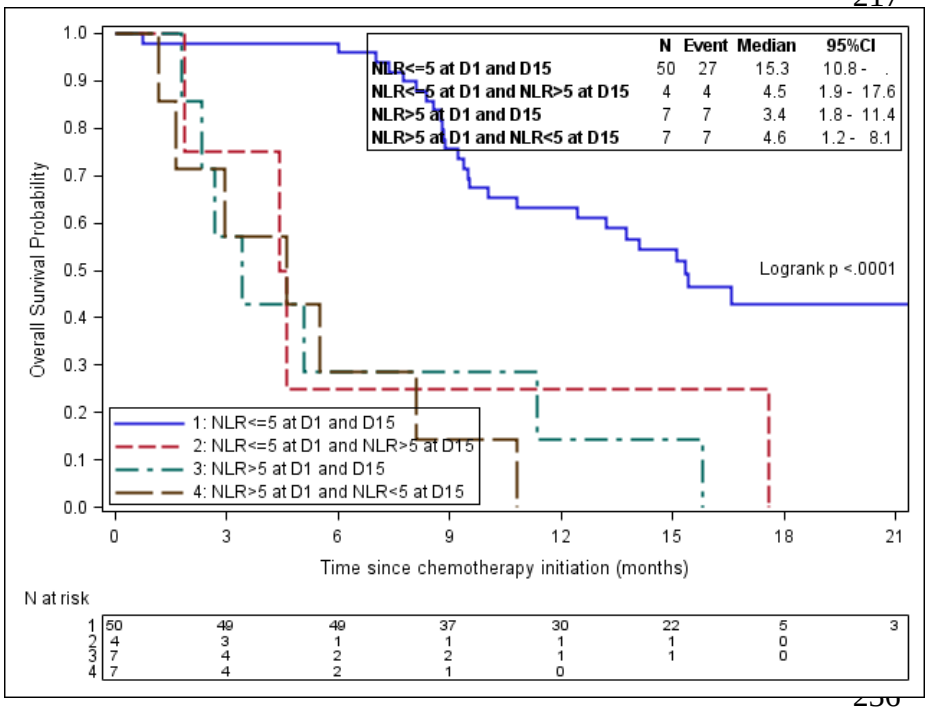
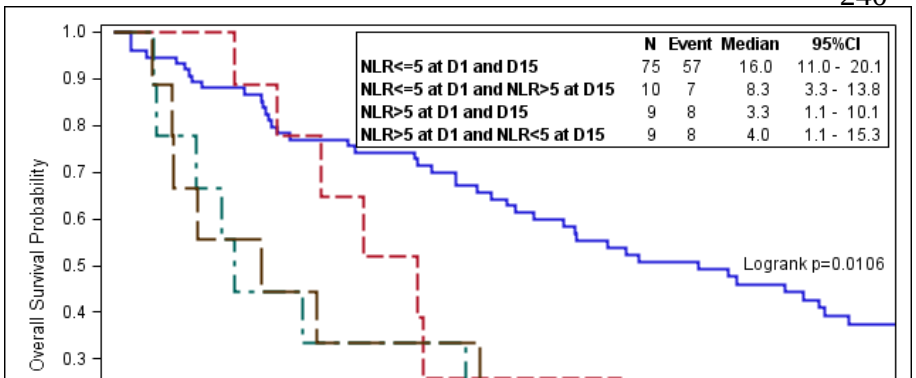


Figure A15. Overall survival according to NLR on day 1 and day 15 of cycle in A) the AFUGEM cohort and B) in the GHPS cohort

A



B

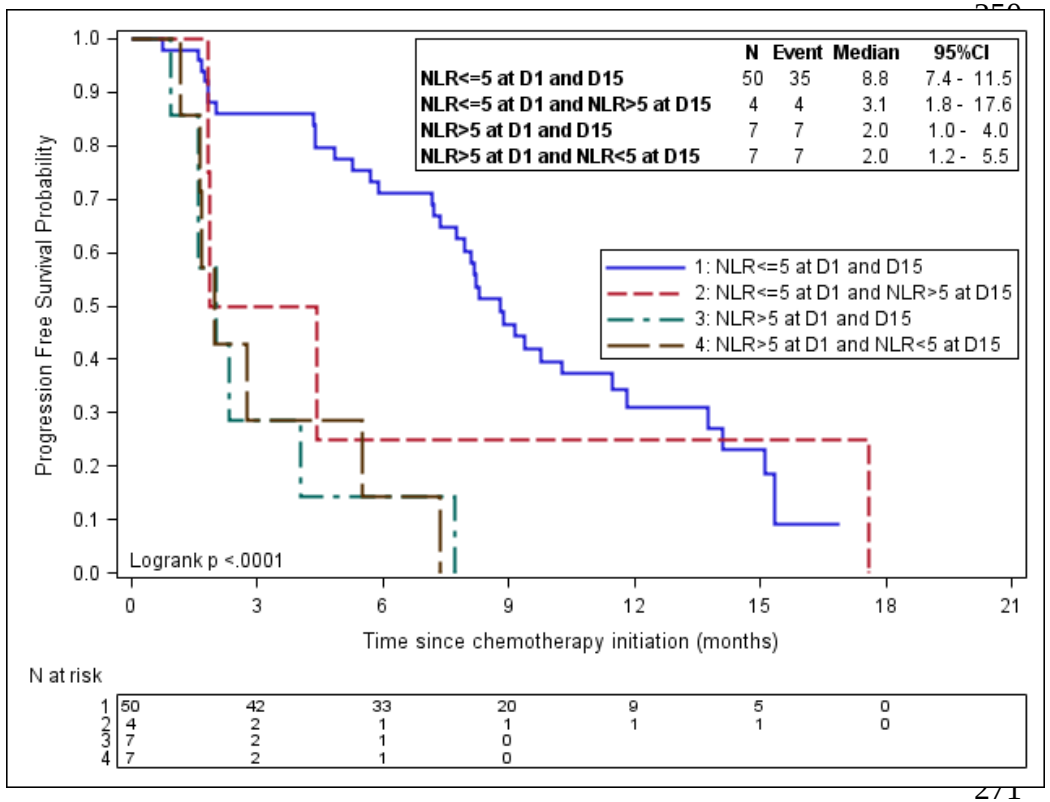


Figure A16. Progression-free survival according to NLR on day 1 and day 15 of cycle in A) the AFUGEM cohort and B) the GHPS cohort

A

B

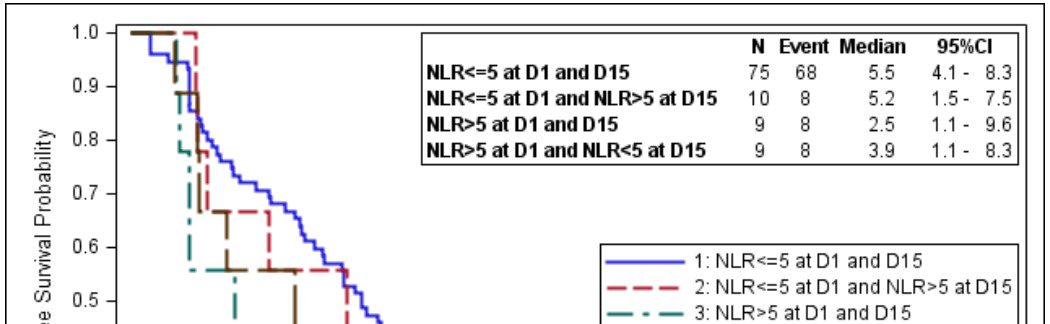
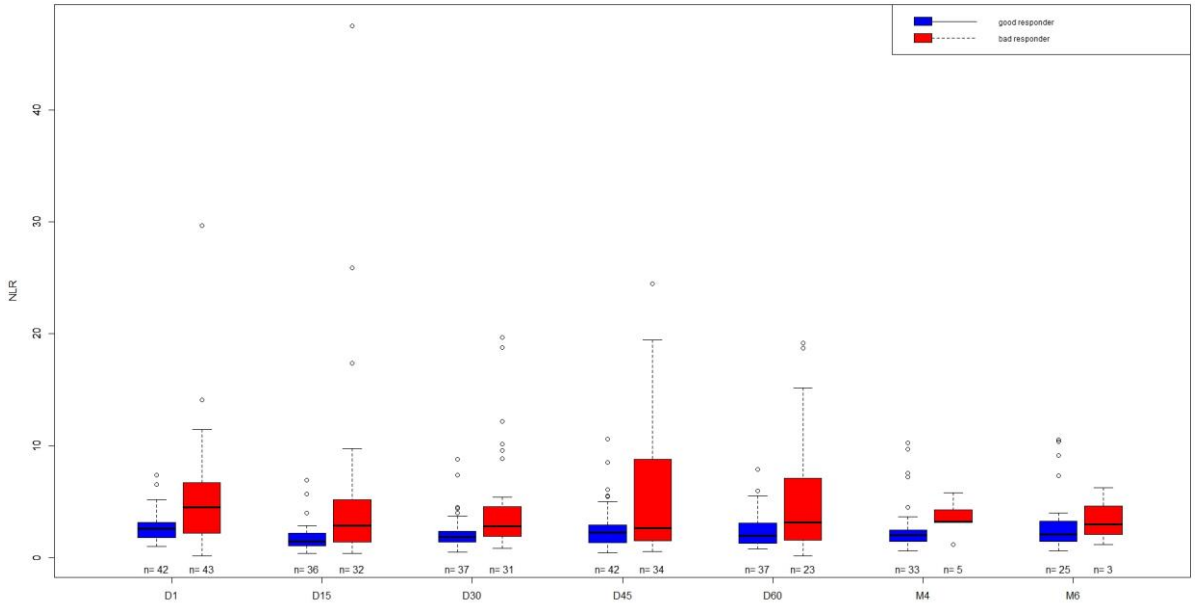

1.0
0.9
0.8
0.7
0.6
0.5
Survival Probability
N Event Median 95%CI
NLR<=5 at D1 and D15 75 68 5.5 4.1 - 8.3
NLR<=5 at D1 and NLR>5 at D15 10 8 5.2 1.5 - 7.5
NLR>5 at D1 and D15 9 8 2.5 1.1 - 9.6
NLR>5 at D1 and NLR<5 at D15 9 8 3.9 1.1 - 8.3
1: NLR<=5 at D1 and D15
2: NLR<=5 at D1 and NLR>5 at D15
3: NLR>5 at D1 and D15

Figure A17. Evolution of NLR over time in A) the AFUGEM cohort and B) the GHPS cohort

A



B

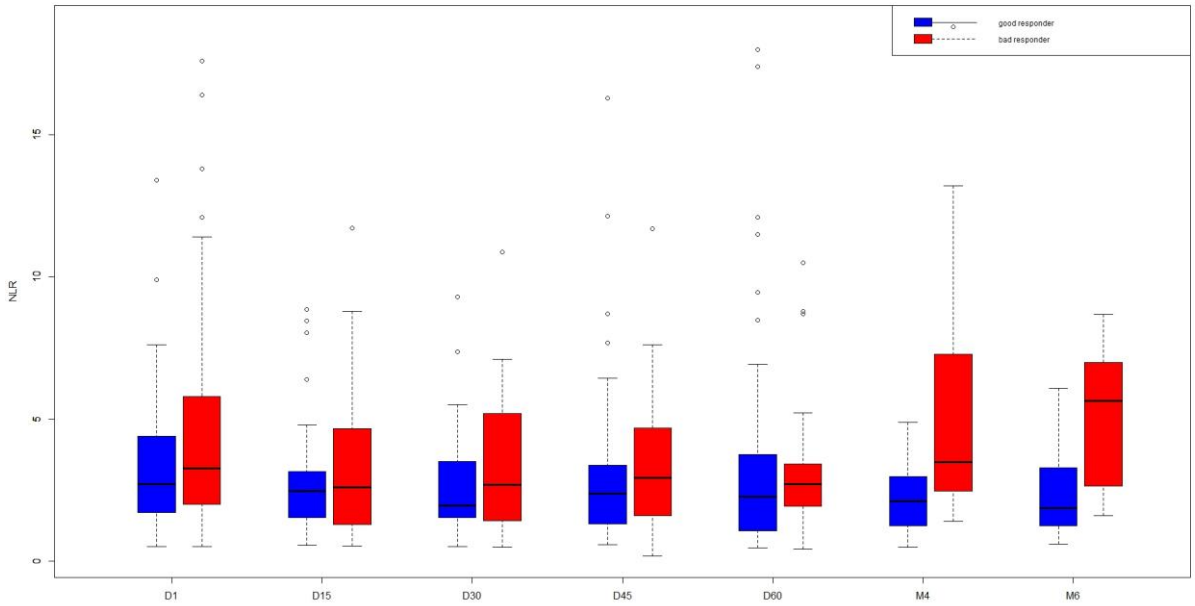
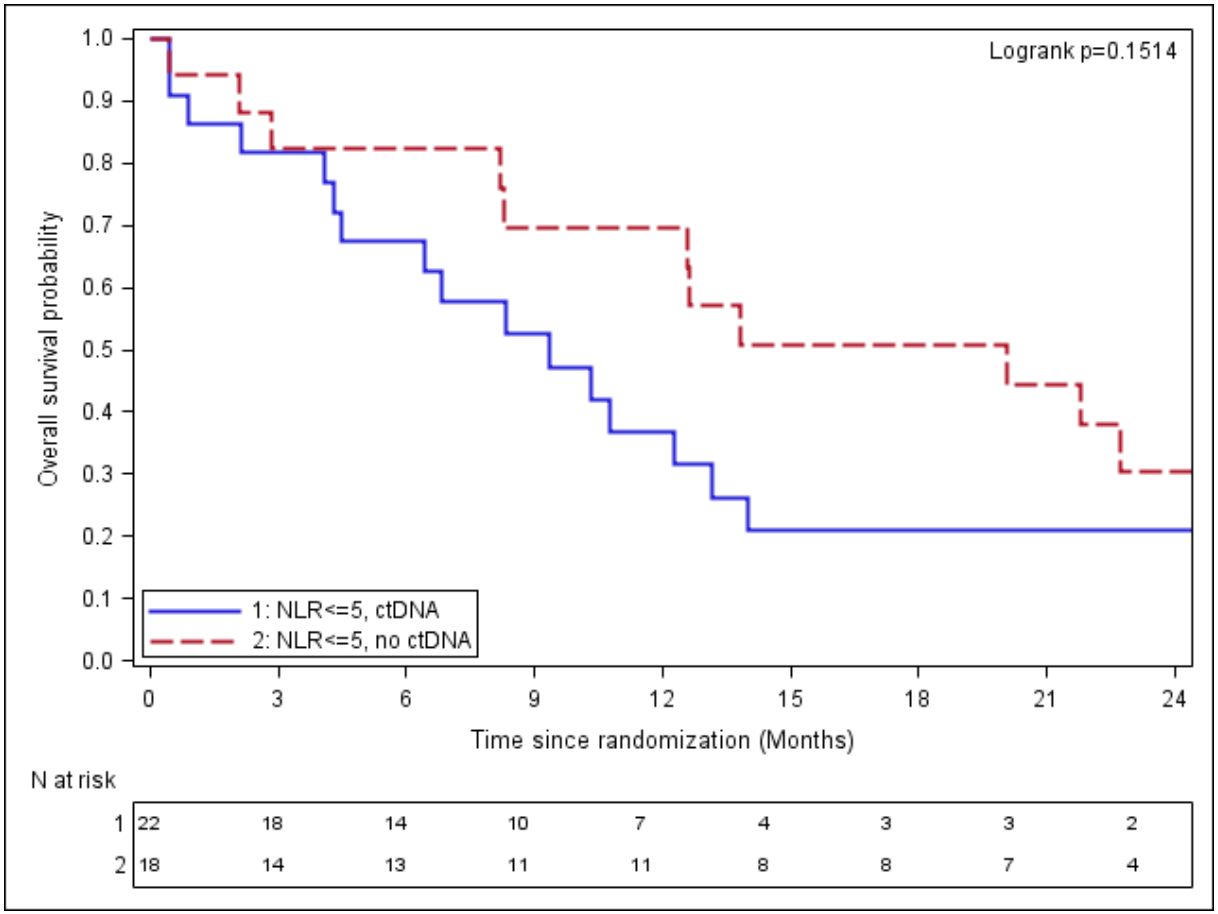


Figure A18. Overall survival in patients with baseline NLR < 5 according to the presence of ctDNA in the GHPS cohort


Logrank p=0.1514
Overall survival probability
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1: NLR<=5, ctDNA
2: NLR<=5, no ctDNA
0
3
6
9
12
15
18
21
24
Time since randomization (Months)
N at risk
1 22 18 14 10 7 4 3 3 2
2 18 14 13 11 11 8 8 7 4

333

Figure A19. Progression-free survival in patients with baseline NLR < 5 according to the presence of ctDNA in the GHPS cohort