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ORIGINAL ARTICLE

Baseline metabolic tumor burden on FDG PET/CT scans predicts outcome in advanced NSCLC patients treated with immune checkpoint inhibitors

Romain-David Seban[1] · Laura Mezquita[2] · Arnaud Berenbaum[1] · Laurent Dercle[3] · Angela Botticella[4] · Cécile Le Pechoux[4] · Caroline Caramella[5] · Eric Deutsch[4,6,7] · Serena Grimaldi[1] · Julien Adam[8] · Samy Ammari[5] · David Planchard[2] · Sophie Leboulleux[1] · Benjamin Besse[2,7]

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Abstract

Purpose We aimed to evaluate if imaging biomarkers on FDG PET are associated with clinical outcomes in patients with advanced non-small cell lung cancer (NSCLC) treated with immune checkpoint inhibitors (ICIs).

Methods In this retrospective monocentric study, we included 109 patients with advanced NSCLC who underwent baseline FDG PET/CT before ICI initiation between July 2013 and September 2018. Clinical, biological (including dNLR = neutrophils/[leukocytes minus neutrophils]), pathological and PET parameters (tumor SUVmax, total metabolic tumor volume [TMTV]) were evaluated. A multivariate prediction model was developed using Cox models for progression-free survival (PFS) and overall survival (OS). The association between biomarkers on FDG PET/CT and disease clinical benefit (DCB) was tested using logistic regression.

Results Eighty patients were eligible. Median follow-up was 11.6 months (95%CI 7.7–15.5). Sixty-four and 52 patients experienced progression and death, respectively. DCB was 40%. In multivariate analyses, TMTV > 75 cm^3 and dNLR > 3 were associated with shorter OS (HR 2.5, 95%CI 1.3–4.7 and HR 3.3, 95%CI 1.6–6.4) and absence of DCB (OR 0.3, 95%CI 0.1–0.9 and OR 0.4, 95%CI 0.2–0.9). Unlike TMTV, dNLR was a significant prognostic factor for PFS (HR 1.9, 95%CI 1.1–3.3) along with anemia (HR 1.9, 95%CI 1.2–3.8). No association was observed between tumor SUVmax and PFS or OS.

Conclusion Baseline tumor burden (TMTV) on FDG PET/CT scans and inflammatory status (dNLR) were associated with poor OS and absence of DCB for ICI treatment in advanced NSCLC patients, unlike tumor SUVmax, and may be used together to improve the selection of appropriate candidates.

Keywords Advanced non-small-cell lung carcinoma · Immune checkpoint inhibitors · Fluorodeoxyglucose F18-positron emission tomography computed tomography · Derived neutrophil to lymphocyte ratio

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✉ Benjamin Besse
Benjamin.BESSE@gustaveroussy.fr

[1] Gustave Roussy, Department of Nuclear Medicine and Endocrine Oncology, Université Paris-Saclay, Villejuif, France

[2] Gustave Roussy, Department of Medical Oncology, Thoracic Unit, Villejuif, France

[3] Gustave Roussy Cancer Campus, UMR1015, Université Paris Saclay, Villejuif, France

[4] Gustave Roussy, Department of Radiation Oncology, Villejuif, France

[5] Gustave Roussy, Department of Radiology, Université Paris-Saclay, Villejuif, France

[6] Gustave Roussy Cancer Campus INSERM U1030Radiomics team, 94800 Villejuif, France

[7] Gustave Roussy Drug Development Department (DITEP), Villejuif, France

[8] Gustave Roussy, Department of Pathology, Université Paris-Saclay, Villejuif, France

Introduction

Over the last few years, treatment of advanced non-small cell lung cancer (NSCLC) has improved significantly, especially with the introduction of immune checkpoint inhibitors (ICIs) [1]. Some of these agents specifically target the programmed cell death 1 receptor (PD-1) (pembrolizumab and nivolumab) or the programmed cell death-ligand 1 receptor (PD-L1) (atezolizumab), and have been approved by the EMA and the FDA for treatment of NSCLC [2–6]. Because only a small fraction of patients experiences long-term benefit of ICI, early identification of patients who may benefit from such therapies is an area of intensive investigation [7]. Expression of PD-L1 is the unique companion diagnostic test approved to date, while the value of tumor mutational burden is actively explored [8]. Other potential biomarkers have been proposed, such as serum lactate dehydrogenase (LDH) levels, a measure of the "biological" tumor burden [9], or the number of various immune cell types in peripheral blood [10], such as lymphocytes, eosinophils, and neutrophils which can be used to determine the neutrophil to lymphocyte ratio (NLR) or the derived NLR (dNLR) [11–13].

In the current new era of immunotherapy, baseline imaging biomarkers offer a standardized and reproducible technique for identifying potential appropriate candidates, as highlighted previously with CT scans [14] and 18F-fluorodeoxyglucose positron emission tomography (FDG PET) in solid tumors [15, 16] and Hodgkin lymphoma [17]. In patients with NSCLC, there is a strong rationale that metabolic parameters on pretreatment FDG PET are reliable prognosticators of outcome and survival [18, 19]. Among PET biomarkers, metabolic tumor volume (MTV) and total lesion glycolysis (TLG) both reflect the metabolic tumor burden and are considered to be the strongest prognostic factors, even more so than tumoral maximal standardized uptake values (SUVmax), in NSCLC patients undergoing surgery [19], chemoradiotherapy [20], chemotherapy [21], or targeted therapies [22]. In the case of immunotherapy, this has been demonstrated in a recent study by Kaira et al, who showed that FDG PET predicted efficacy and survival at 1 month after nivolumab [23]. However, the prognostic and predictive value of baseline FDG PET remains unclear in advanced NSCLC patients treated with ICIs.

Although several factors had been linked to the therapeutic efficacy of ICIs, relevant predictive and prognostic biomarkers for estimating the likelihood of effective ICI treatment and overall survival (OS) are still needed [24]. In this study, we aimed to evaluate if imaging biomarkers, particularly the metabolic tumor burden identified in pretreatment FDG PET scans, can predict clinical outcome in advanced NSCLC patients who undergo therapy with an ICI. Secondly, we assessed these imaging biomarkers in combination with biological parameters to identify a means of stratifying the treatment approach for the population that may receive ICIs.

Methods

Patients

We conducted a retrospective study of patients with advanced NSCLC treated with ICIs between July 2013 and September 2018, who underwent a combined imaging protocol of FDG PET/CT, as part of a standard care or in a clinical study at the Gustave Roussy Cancer Center (GRCC), Villejuif, France. The study was approved by the institutional review board (IRB) and waived the requirement for informed consent.

Main inclusion criteria were treatment with a single agent monoclonal IgG targeting PD-1 or PD-L1, and histological or cytological confirmation of NSCLC, stage IV or IIIB, which was ineligible for local therapy. Patients were excluded if they delay between FDG PET/CT, and the first ICI perfusion was > 6 weeks (n = 22), were lost to follow-up (n = 3), or had other primary malignancies (n = 4). Demographic, clinical, pathological, biological, and molecular data were collected. The inclusion criteria for blood samples assessment before ICIs therapy was 28 days.

FDG PET/CT protocol

Prior to FDG PET scans, patients fasted for at least 6 h, and blood glucose levels were confirmed to be <180 mg/dL. Patients were injected according to current guidelines with FDG (median activity 255 MBq [range, 106–446] – median 3.0 MBq/kg), and images were acquired 60 min later (median 60 min). A CT scan was obtained initially, followed by a PET scan, performed from the skull base to the proximal femur. Three PET/CT scanners were used: General Electric Discovery 690 (GE Healthcare, Waukesha, WI) (63 patients), Philips Gemini TF TOF 16 PET/CT scanner (10 patients), and both with LYSO-based detectors and Siemens PET/CT Biograph 40 with LSO crystal (7 patients). PET images were reconstructed using iterative algorithms (GE Discovery 690: OSEM algorithm, time of flight – TOF reconstruction, matrix 256 × 256, 3 iterations, 16 subsets, post-filter 6.3 mm; Philips Gemini TF 16: OSEM algorithm, time of flight – TOF reconstruction, matrix 512 × 512, 3 iterations, 8 subsets, post-filter 8 mm; Siemens Biograph 40: PSF algorithm, matrix 168 x 168, 3 iterations, 21 subsets, post-filter 5 mm). Each de-identified and anonymized patient was analyzed on-site by a pair of experienced nuclear medicine physicians who regularly

perform and review FDG PET scans according to the same protocol.

Measurement of biological and imaging biomarkers and PD-L1 expression

All hypermetabolic metastatic lesions were selected for analysis, while hypermetabolic foci explained by inflammatory or physiologic activity were excluded. For each metastatic lesion, SUVmax and MTV values were measured. SUV was calculated in a pixel as (radioactivity)/(injected dose/body weight). MTV was measured with setting a margin threshold of 42% of SUVmax [25]. All values of SUVmax and MTV were automatically measured by the analysis software for each lesion. A patient's SUVmax was defined as the highest SUVmax recorded among all lesions detected, and total MTV (TMTV) was defined as the sum of all lesions.

Complete blood cell counts, LDH, and albumin levels at baseline before ICI treatment (within 28 days before the first treatment) were extracted from electronic medical records. The cutoff for dNLR was > 3 (based on the cutoff from the largest published study with ICIs [26]), and the upper limit of normal (ULN) was defined according the limit of our biochemical laboratory (LDH: ULN = 248 UI/L; albumin: ULN = 37 g/L) or widely applied thresholds (anemia if Hb ≤ 12 g/dL, neutrophilia if neutrophils > 7×10^9/L and thrombocytosis if platelets > $400x10^9$/L).

Tumor PD-L1 expression was assessed locally by immunohistochemical staining with six PD-L1 immunohistochemistry (IHC) assays (22C4, 28–8, QR1, E1L3N, SP142, and SP263), in pretreatment and archival biopsy samples. Samples with membrane staining in ≥ 1% of tumor and/or immune cells were considered positive.

Outcomes

Progression-free and overall survival

The primary end point analysis was to develop and validate a multivariate model using pretreatment FDG PET imaging to predict outcome. Progression-free survival (PFS) was defined as the time from the first ICI perfusion to disease progression or death from any cause, and OS was defined as the time from the first ICI perfusion to the date of death due to any cause or of censoring at the last time the patient was known to be alive. Follow-up was calculated from the date of the initial PET/CT to the date of the last clinical consultation. Assessment of outcome was blinded.

Response evaluation

The secondary end point analysis was to evaluate the accuracy of baseline FDG PET for disease clinical benefit (DCB) classification. Radiological assessments with CT scans were performed every 6 to 8 weeks per RECIST (response evaluation criteria in solid tumors) v1.1.

Patients who achieved objective partial or complete response (PR or CR) at any time during the treatment or stable disease (SD) after 6 months were defined as having DCB. All other patients were classified as being without DCB.

Statistical analysis

Continuous variables were dichotomized at their median value. Values of biomarkers between patients with or without DCB, patients with or without corticosteroids at baseline, and patients with low and high PD-L1 expression were

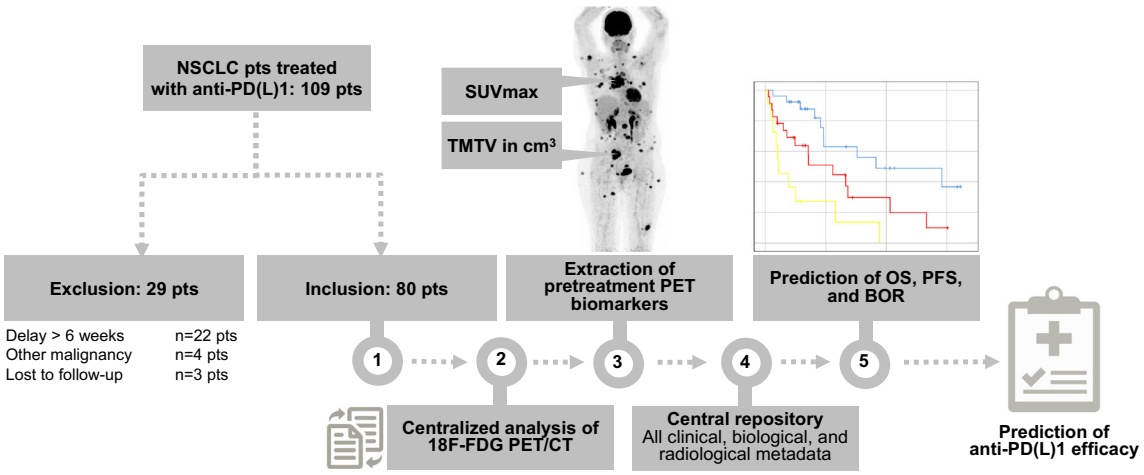


Fig. 1 Flow chart. Abbreviations: *NSCLC* (non-small cell lung cancer), *PD/PD-L1* (programmed cell death-1/ programmed death-ligand 1), *FDG PET/CT* (18F-fluorodeoxyglucose positron emission tomography/ computed tomography), *SUVmax* (maximum standardized uptake value), *TMTV* (total metabolic tumor volume), OS (overall survival), PFS (progression-free survival), *BOR* (best overall response)

compared using the nonparametric Kruskal–Wallis test. Tests for continuous variables dealt both with the binary form and the continuous form (p trend). The prognostic value for survival of all pretreatment imaging biomarkers was studied with Cox models for survival. Multivariate analyses were performed using Cox proportional hazard regression models in a stepwise manner for independent significant factors. Spearman's rank correlation coefficients were calculated to assess the relationships between parameters. Factors associated with DCB were tested with logistic regression with the backward elimination method in univariate and multivariate analyses. P values were adjusted to account for the multiple comparisons issue using the Holm–Bonferroni method (adjusted p). A significance threshold of 5% was used. Analyses were performed with PASW Statistics for Windows (version 25. Chicago: SPSS Inc).

Results

Patient characteristics

Of 109 consecutive patients with FDG PET before receiving an ICI who were screened, 80 were included, 77 were identified from standard care evaluation, and 3 patients were included in clinical trials (Fig. 1). Baseline patient characteristics are summarized in Table 1. In most of the patients, blood samples were collected in the 7 days before the ICIs beginning (mean

Table 1 Baseline characteristics

	n (%)	Median (range)
Clinical Parameters *(Threshold)*		
Age (years)	-	61.9 (34.2–84.8)
Sex (male/female)	56 (70)/24 (30)	-
Body mass index (kg/m^2)	-	23.2 (13.2–39.5)
Performance status (*ECOG ≥ 2)*	-	1 (0-3)
Smoking status (current/former/never/unknown)	27 (34)/45 (56)/5 (6)/3 (4)	
Stage groups: III/IV	19 (24)/61 (76)	
Treatment		
Nivolumab/pembrolizumab/atezolizumab	54 (68)/21 (26)/5 (6)	-
Number of prior therapies	-	2.0 (0–6)
Histology		
SCC/ADK/other	28 (35)/46 (57)/6 (8)	-
Molecular parameters		
Main mutation: KRAS/EGFR/ALK/other†/WT/unknown	12 (15)/2 (2.5)/2 (2.5)/15 (19)/27 (34)/22 (27)	-
PD-L1 ≥ 1%/ < 1% / unknown	24 (30)/17 (21)/39 (49)	-
Biology		
Neutrophils *(> 7.10^9/L)*	*36 45)*	6.7 (2.1–25.6)
dNLR *(> 3)*	*40 (50)*	3.2 (0.4–12.2)
Hemoglobin *(≤ 12 g/dL)*	*38 (48)*	11.9 (7.6–15.1)
Platelets *(> 400.10^9/L)*	*21 (26)*	305 (21–1157)
LDH *(> 248UI/L)*	*28 (35)*	231 (92–1157)
Albumin *(≤ 37 g/L)*	*38 (48)*	37 (24–50)
Tumor imaging – PET		
Number of metastatic sites *(> 3)*	*22 (28%)*	3 (0–8)
Metastatic site: liver/brain/bone/adrenal	15 (19)/22 (27)/32 (40)/16 (20)	–
Tumor SUVmax *(> 12.8)*	*40 (50)*	12.8 (4.7–50.1)
TMTV *(> 75 cm3)*	*40 (50)*	75.0 (4.6–670.8)

†Other: BRAF, MET, BRCA1, HER-2, PI3KCA, TP53, CDKN2A, STK11.

**Patients with PD-L1 status available*

Abbreviations: *ECOG* (eastern cooperative oncology group), *SCC* (squamous cell carcinoma), *ADK* (adenocarcinoma), *WT* (wild-type), *PD-L1* (programmed death-ligand 1), *dNLR* (derived neutrophils to lymphocytes ratio), *LDH* (lactate dehydrogenase), *PET* (positron emission tomography), *SUVmax* (maximum standardized uptake value), *TMTV* (total metabolic tumor volume), *ULN* (upper limits of normal)

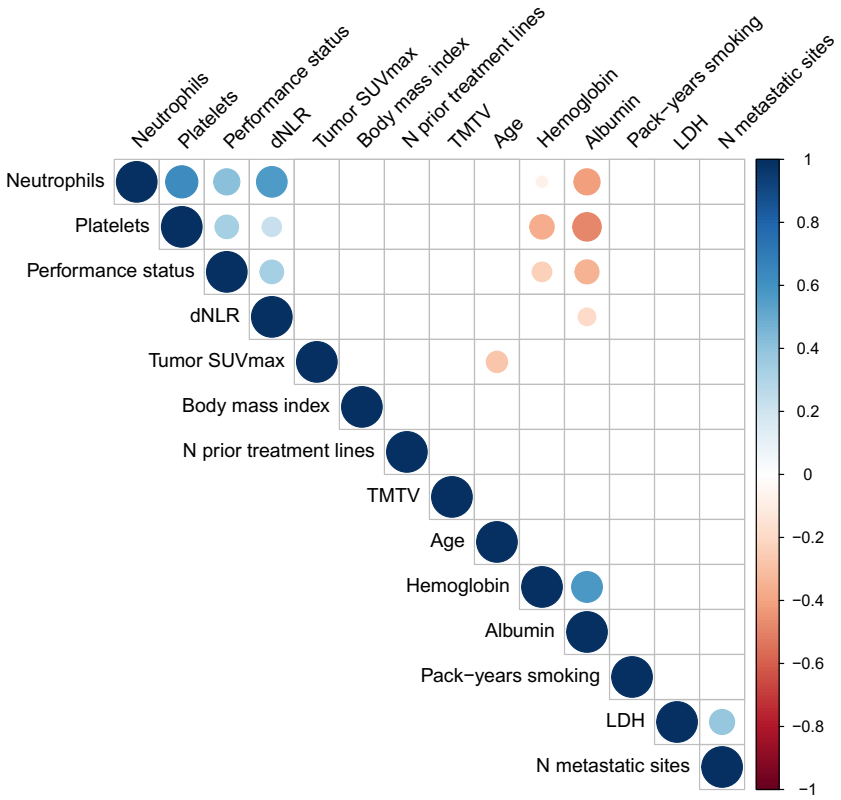


Fig. 2 Correlogram: correlations between TMTV, tumor SUVmax and clinical or biological variables (Spearman's coefficient) Abbreviations: *dNLR* (derived neutrophils to lymphocytes ratio), *LDH* (lactate dehydrogenase), *PET* (positron emission tomography), *SUVmax* (maximum standardized uptake value), *TMTV* (total metabolic tumor volume), *N metastatic sites* (number of metastatic sites)

time interval 4.4 days; minimum 0; maximum 26). In terms of disease, 24% had stage III NSCLC and 76% had stage IV before ICI treatment. Patients had a median of three metastatic sites (range, 0–8), 15 patients (19%) had liver metastasis, and 22 (27%) had brain metastasis. Patients had received a median of two lines of therapy before ICI treatment (range 0–6) (characteristics of patients with ICI for first-line therapy and patients with ICI for subsequent line therapy are provided in Supplemental Table 1). Nineteen patients (24%) received prior thoracic radiotherapy and 28 (35%) had prior platinum-based chemotherapy. Nine patients were treated with > 10 mg of prednisone-equivalent dose at baseline (type of

Table 2 Prognostic significance of biomarkers for progression-free survival and overall survival in univariate and multivariate analyses

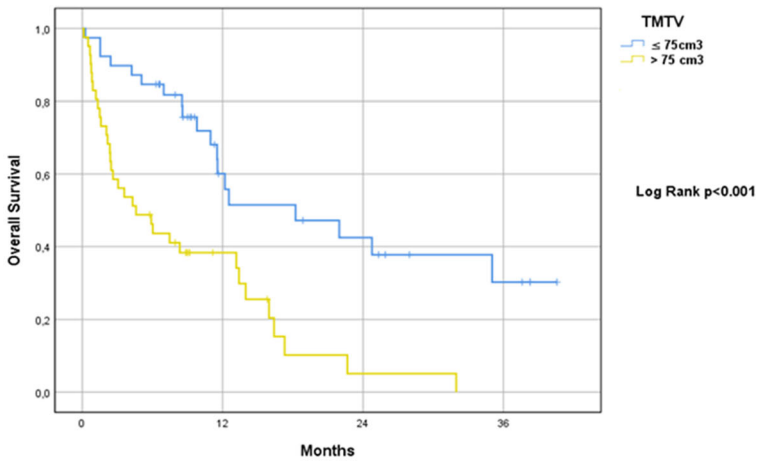
	Progression-free survival			Overall survival			
n = 80 patients	Univariate		Multivariate	Univariate		Multivariate	
Variable	Adjusted *p*	HR (CI 95%)	p	HR (CI 95%)	Adjusted *p*	HR (CI 95%)	*p*	HR (CI 95%)
PS (≥ 2 vs < 2)	0.23	0.9 (0.7–1.1)	–	–	0.02	2.9 (1.4–6.0)	0.23	1.6 (0.7–3.4)
Histology (SCC vs non-SCC)	0.21	0.7 (0.4–1.2)	–	–	0.43	1.3 (0.7–2.2)	-	-
Smokers (never vs always)	0.09	0.4 (0.2–1.1)	–	–	0.75	1.2 (0.4–3.9)	-	-
dNLR (> 3 vs ≤ 3)	0.02	2.3 (1.4–4.1)	0.007	1.9 (1.1–3.3)	0.001	3.6 (1.8–7.0)	0.001	3.3 (1.6–6.4)
Hemoglobin (≤ 12 vs > 12 g/dL)	0.04	2.2 (1.3–3.8)	0.02	1.9 (1.2–3.8)	0.06	1.8 (0.9–3.1)	-	–
LDH (> 248 vs ≤ 248 UI/L)	0.13	1.1 (0.8–1.3)	–	–	0.09	1.2 (0.8–2.3)	-	–
N metastatic sites (> 3 vs ≤ 3)	0.24	0.9 (0.8–1.1)	–	–	0.07	2.1 (1.2–3.8)	-	–
Liver metastasis (yes vs no)	0.39	1.3 (0.7–2.1)			0.26	1.4 (0.8–2.7)	-	–
Tumor SUVmax (> 12.8 vs ≤ 12.8)	0.35	0.8 (0.5–1.3)	–	–	0.63	0.9 (0.5–1.5)	-	–
TMTV (> 75 vs ≤ 75 cm^3)	0.25	1.0 (0.9–1.1)	-	–	0.001	3.1 (1.7–5.7)	0.004	2.5 (1.3–4.7)

Abbreviations: *PS* (performance status), *SCC* (squamous cell carcinoma), *dNLR* (derived neutrophils to lymphocytes ratio), *LDH* (lactate dehydrogenase), *N metastatic sites* (number of metastatic sites), *SUVmax* (maximum standardized uptake value), *TMTV* (total metabolic tumor volume), *HR* (hazard-ratio), *CI* (confidence interval), *ULN* (upper limits of normal)

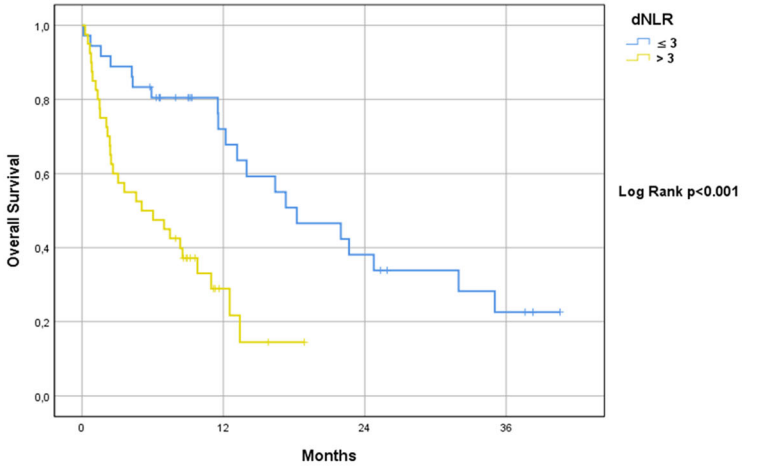
corticoids, daily dose and reason are detailed in Supplemental Table 2), with clinical, biological or imaging characteristics similar to those in patients without corticosteroids (Supplemental Table 3). After a median follow-up of 11.6 months (95%CI 7.7–15.5), 64 (80%) and 52 (65%) patients had experienced progression and death, respectively. The distribution of PET parameters is described in supplemental Fig. 1. Thresholds, corresponding to median values of the whole cohort (80 patients), were: SUVmax > or ≤ 12.8 and TMTV > or ≤ 75 cm^3.

Correlation between biomarkers

Correlations between PET biomarkers, extracted from tumor lesions and variables of interest, are presented in Fig. 2. We found that tumor SUVmax and TMTV didn't correlate significantly with each other. Furthermore, tumor SUVmax or TMTV (continuous variable) were not correlated with any clinical or biological variables, with one exception (association between tumor SUVmax and age: rank [rho] = 0.3; $p < 0.05$).



Number at risk								
Low TMTV	39	33	14	12	9	5	4	0
High TMTV	41	18	9	2	1	0	0	0
Time (months)	0	6	12	18	24	30	36	42



Number at risk								
Low dNLR	36	28	17	12	9	6	4	0
High dNLR	40	20	4	1	0	0	0	0
Time (months)	0	6	12	18	24	30	36	42

Fig. 3 Association of TMTV and dNLR with overall survival Kaplan-Meier curves for OS in all patients (n = 80). Curves for OS stratified according to TMTV (left) and dNLR (right) Abbreviations: *TMTV* (total metabolic tumor volume), *dNLR* (derived neutrophils to lymphocytes ratio)

Univariate and multivariate analyses: PFS and OS

Median PFS was 2.5 months (95%CI 1.6–3.3). In univariate analysis, high dNLR (> 3) and anemia (hemoglobin < 12 g/dL) were significantly associated with poor PFS (adjusted p values < 0.05) (Table 2). In multivariate analysis, high dNLR and anemia remained independent statistically significant prognostic factors for PFS (HR 1.9, 95%CI 1.1–3.3 and HR 1.9, 95%CI 1.2–3.8, respectively) (Table 2). Tumor SUVmax (> 12.8) and TMTV (> 75 cm^3) were not correlated with PFS, LDH levels (> 248 UI/L), number of metastatic sites (> 3), or the presence of liver metastasis.

Median OS was 9.2 months (95%CI 6.2–12.2). In univariate analysis, performance status (ECOG ≥ 2), high dNLR (> 3) and high TMTV (> 75 cm^3) were significantly associated with poor OS (adjusted p value < 0.05) (Table 2, Fig. 3). In multivariate analysis, high TMTV and high dNLR remained independent statistically significant prognostic factors for OS (HR 2.5, 95%CI 1.3–4.7 and HR 3.3, 95%CI 1.6–6.4, respectively) (Table 2). Despite a clear trend in univariate analysis, the number of metastatic sites (> 3), LDH levels and anemia were not statistically significant prognostic factors for OS (adjusted p value > 0.05 and < 0.1, respectively). Tumor SUVmax and liver metastatic involvement were not correlated with OS.

DCB classification: patients with versus without clinical benefit

According to the best overall response with RECIST 1.1, DCB was achieved among 40% patients treated with ICI (32 patients: 0 with CR, 19 with PR, and 13 with SD > 6 months). TMTV, PS, and dNLR were significantly lower and Hb significantly higher ($p < 0.05$) in patients with DCB compared to patients without DCB (supplemental Table 4, supplemental Fig. 2). Tumor SUVmax appeared higher among patients with DCB but was not statistically significant (mean: 16.7 versus 13.2; $p = 0.18$). In multivariate logistic regression, high TMTV and high dNLR remained independent factors for poor DCB (odds ratio [OR] 0.3, 95%CI 0.1–0.9 and OR 0.4, 95%CI 0.2–0.9, respectively) (table 3). The number of metastatic sites (≤3) and the absence of liver metastasis were not associated with DCB.

Determining a metabolic score

We developed a score combining the TMTV and dNLR, both independent factors for OS and DCB in multivariate analysis (supplemental Fig. 3) and used it to stratify the population into three groups; a good prognosis group for TMTV ≤ 75 cm^3 and dNLR ≤ 3 (n = 24, 30%), intermediate prognosis group for TMTV > 75 cm^3 or dNLR > 3 (n = 33, 41%), and poor prognosis group for TMTV > 75 cm^3 and dNLR > 3 (n = 23, 29%). Median OS was 35.0 months (95%CI 14.6–55.4) for the good prognosis group versus 12.5 months (95%CI 6.6–18.5) for the intermediate prognosis group versus 2.4 months (95%CI 1.9–2.9) for the poor prognosis group ($p < 0.001$) (Fig. 4). Median PFS was 9.8 months (95%CI 5.2–14.4) for the good prognosis group versus 2.7 months (95%CI 1.7–5.8) for the intermediate prognosis group versus 1.4 months (95%CI 0.8–2.0) for the poor prognosis group ($p < 0.001$). The metabolic score also correlated with response. The good prognosis group was associated with DCB, with an OR of 7.9 (95%CI, 2.0–30.7, $p = 0.003$) (supplemental Table 5), which

Table 3 Univariate and multivariate analyses of the relationship between patient characteristics and disease clinical benefit

	Disease clinical benefit n = 80 patients			
Logistic regression	Univariate	Multivariate		
Variable	adjusted p	OR (CI 95%)	p	OR (CI 95%)
PS (≥ 2 vs < 2)	0.045	0.2 (0.04–0.9)	0.14	0.3 (0.1–1.5)
Histology (SCC vs non-SCC)	0.39	1.5 (0.6–3.8)	-	-
Smokers (never vs always)	0.33	3.0 (0.3–28.4)	-	-
dNLR (> 3 vs ≤ 3)	0.01	0.3 (0.1–0.8)	0.048	0.4 (0.2–0.9)
Hemoglobin (≤ 12 vs > 12 g/dL)	0.08	0.4 (0.2–1.1)	-	-
LDH (> 248 vs ≤ 248 UI/L)	0.9	1.0 (0.4–2.7)	-	-
N metastatic sites (> 3 vs ≤ 3)	0.11	0.4 (0.1–1.2)	-	-
Liver metastasis (yes vs no)	0.19	0.5 (0.2–1.4)	-	-
Tumor SUVmax (> 12.8 vs ≤ 12.8)	0.12	2.0 (0.8–5.1)	-	-
TMTV (> 75 vs ≤ 75 cm3)	0.01	0.3 (0.1–0.8)	0.045	0.3 (0.1–0.9)

Abbreviations: *PS* (performance status), *SCC* (squamous cell carcinoma), *dNLR* (derived neutrophils to lymphocytes ratio), *LDH* (lactate dehydrogenase), *N metastatic sites* (number of metastatic sites), *SUVmax* (maximum standardized uptake value), *TMTV* (total metabolic tumor volume), *OR* (odds-ratio), *CI* (confidence interval), *ULN* (upper limits of normal)

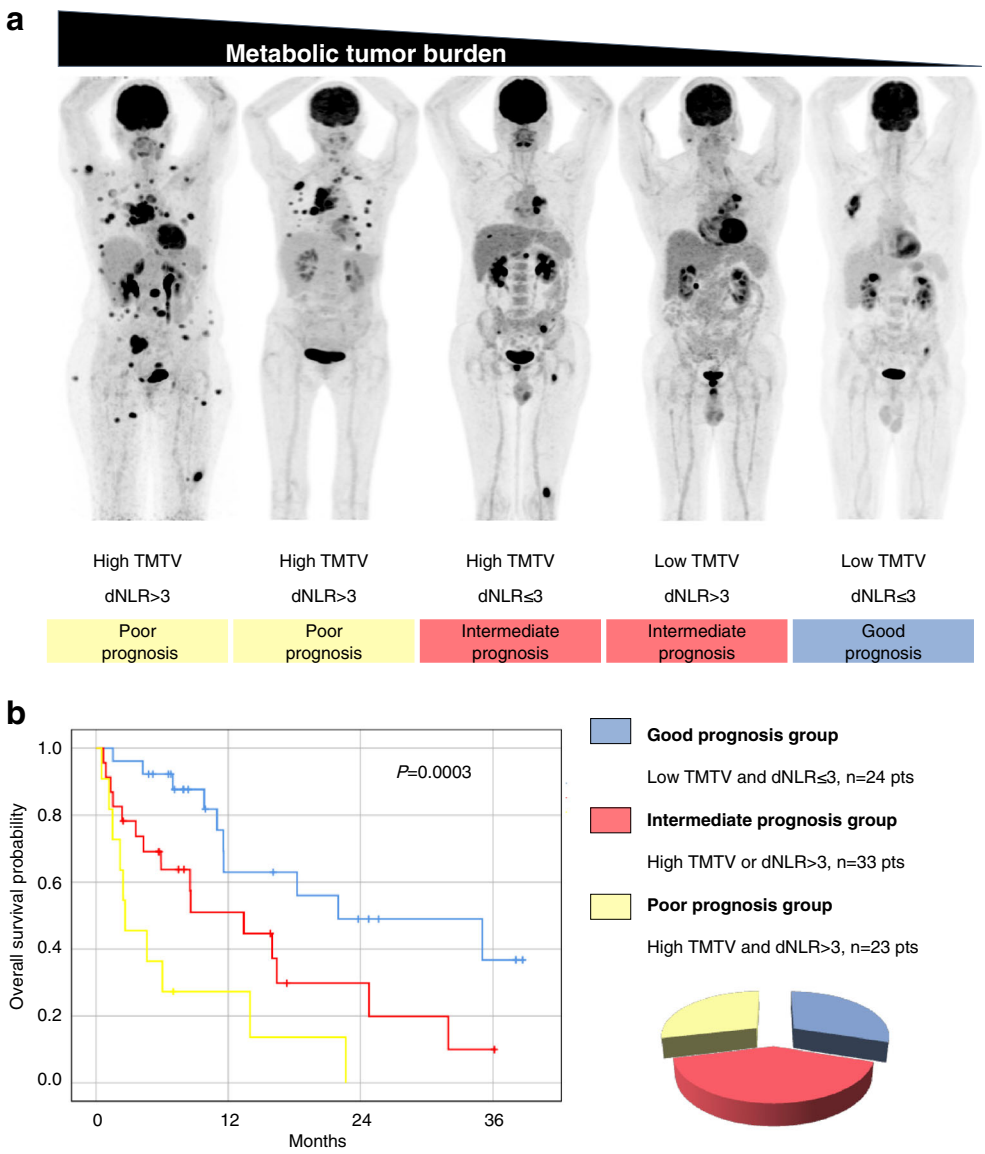


Fig. 4 Prognostic imaging biomarkers. **A.** Illustration of low versus high total metabolic tumor volume using maximal intensity projection on FDG-PET images of five patients. **B.** Kaplan–Meier curve of overall survival (OS) according to a metabolic score combining TMTV and dNLR The combined score comprising two binary risk variables was defined as follows: 0 = good prognosis group (TMTV ≤ 75 cm3 AND dNLR ≤ 3); 1 = intermediate prognosis group (TMTV > 75 cm3 OR dNLR > 3); left = high TMTV and low dNLR; right = low TMTV and high dNLR; 2 = poor prognosis group (TMTV > 75 cm3 AND dNLR > 3).

was even higher than those determined with TMTV or dNLR alone (OR 4.6 and 3.5, respectively).

PD-L1 expression: patients with PD-L1 positive versus negative tumor

A total of 41 patients (51%) were evaluable for PD-L1 (available data). The high rate of missing PD-L1 status was mainly due to the fact that it was not necessarily requested for ICI prescription. Clinical, biological and imaging parameters, including FDG PET/CT, in patients with and without PD-L1 protein expression (≥ and < 1%, respectively) are shown in supplemental Table 6. PD-L1 expression was significantly higher in patients with high tumor SUVmax (median 13.7 versus 10.9; $p = 0.02$), dNLR ($p = 0.03$), and LDH > ULN ($p < 0.01$). Rather, there was no association between TMTV and PD-L1 expression ($p = 0.18$).

Discussion

Our present study indicates that pretreatment metabolic tumor burden as measured by TMTV on FDG PET has a high potential value in predicting OS and DCB in advanced NSCLC patients intended for treatment with ICI. Patients with high metabolic tumor burden (TMTV > 75 cm^3) were associated with shorter median OS and non-DCB. Moreover, we showed that TMTV remains the best biomarker for predicting outcome among factors reflecting the tumor burden (LDH and number of metastatic sites) and beyond metastatic sites, e.g., liver involvement, known to be associated with reduced responses and PFS in NSCLC patients with anti-PD-1 therapy [27]. This discriminative and prognostic application of TMTV has been used in the past to predict the outcome of other NSCLC treatments, including before surgery [19], radio-chemotherapy [20], chemotherapy [28], and targeted therapy [22]. Our study

strengthens these finding extending them to NSCLC patients receiving ICI treatment. We propose that it should be taken into consideration when selecting appropriate candidates in future clinical trials or studies involving ICIs.

Surprisingly, in our cohort, baseline tumor SUVmax did not provide any prognostic information. Although TMTV is thought to be the most reliable prognostic biomarker, tumor SUVmax is also considered, to a lesser extent, as a strong prognosticator of outcome and survival among patients with NSCLC, regardless of treatment received or disease stage [29–32]. Furthermore, in routine clinical practice, the highest SUVmax is used as a reference for subsequent response evaluation, such as in the EORTC criteria [33–35]. Finally, SUVmax is a metric that is easy to measure, and several studies have also demonstrated that high SUVmax significantly correlates with tumor cell proliferation [36] and is associated with poor prognosis [37, 38].

Combination of multiple biomarkers could be an innovative way to improve both prediction and prognostic accuracy. We developed a score, combining the dNLR, which might reflect the accumulation of inflammatory factors with a negative impact on disease control and survival [9], with TMTV at baseline before ICI, stratifying the population into three prognostic groups. Our findings suggest a greater impact for prognosticating OS and DCB when combining TMTV and dNLR in this promising score (Fig. 4), which need to be validated in large, independent and prospective cohort.

Our findings reinforce the belief of a paradigm shift in tumor metabolism evaluation for ICI assessment [39]. ICIs and conventional cancer treatments, including cytotoxic chemotherapy and targeted therapy, are acting through distinct mechanisms [40] (immunomodulatory effects and direct cancer cell-killing activities), which could impact tumor metabolism differently. Interestingly, we found a markedly increased SUVmax in tumors having a high PD-L1 expression, confirming the direction of previous studies in patients with surgically resected pulmonary carcinoma, through GLUT-1 (glucose transporter-1) and HIF-1 α (hypoxia-inducible factor 1α) overexpression [41–43], and with EGFR-mutant NSCLC treated with TKI-therapy [44]. One hypothesis could be that increased glycolytic metabolism in PD-L1 positive tumors is related to an enhanced immune infiltrate in the tumor microenvironment, such as TILs (tumor infiltrating lymphocytes) in surgically resected NSCLCs [45].

The main limitations of this study include its retrospective nature and the relatively small sample size. Although corticosteroids therapy at baseline have been correlated with poor outcomes under IO [46], a recent publication has revealed that probably is more related to the palliative condition of the patients [47], and not as independent negative factor. Based on this, and taking into consideration the small number of patients treated with steroids in our work, we did not exclude this population. In addition, our methodology for tumor volume measurements, which had been used in several previous studies [24, 33], presents some difficulties in some tumor sites, notably the brain, especially owing to physiological uptake of normal brain tissues. In the fraction of patients with brain metastasis (27%), the tumor burden might be underestimated. However, if we had excluded such patients, our cohort may not have been representative of the real-life patient population eligible for immunotherapy, particularly for advanced NSCLC. In addition, we acquired images on three different PET devices, which could influence the measurement of PET features, but also shows the generalizability of our model to different centers and devices. Finally, the prognostic value of PD-L1 expression could not be investigated and balanced against other potential biomarkers since it was only available in 51% of the cohort, mainly due to the fact that it was not necessarily requested for ICI prescription, and, given that the SP142 PD-L1 IHC assay exhibits fewer stained tumor cells compared with other antibodies, the percentage of PD-L1–stained tumor cells were not comparable [48].

Conclusions

Baseline high TMTV on FDG PET was associated with poor OS and no-DCB following ICI therapy in advanced NSCLC patients, in contrast to the tumor SUVmax or the number of metastatic sites that were not. Combined with biological prognostic factors, as well as pretreatment dNLR, which is correlated with all the investigated outcomes, FDG PET can potentially improve the selection of candidates for ICI and identify patient groups with markedly different prognoses.

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2) All authors revised it critically for important intellectual content.
3) All authors approved the version to be published.
4) All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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- **R-D S, A.B, L.D, A.B, C.C, E.D, S.G, J.A, S.A, S.L**: The authors declare that they have no conflict of interest.

- **L.Mezquita:**

  Consulting, advisory role: Roche Diagnostics

  Lectures and educational activities: Bristol-Myers Squibb, Tecnofarma, Roche, AstraZeneca

  Travel, Accommodations, Expenses: Chugai

- **C.Le Pechoux**:

- **Consulting advisory role**: Amgen, Astra Zeneca, Nanobiotix, Roche
- **Lectures and educational activities**: Astra Zeneca, Lilly

- **D. Planchard**:

- **Consulting, advisory role or lectures**: AstraZeneca, Bristol-Myers Squibb, Boehringer Ingelheim, Celgene, Daiichi Sankyo, Eli Lilly, Merck, MedImmune, Novartis, Pfizer, prIME Oncology, Peer CME, Roche. Honoraria: AstraZeneca, Bristol-Myers Squibb, Boehringer Ingelheim, Celgene, Eli Lilly, Merck, Novartis, Pfizer, prIME Oncology, Peer CME, Roche
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Informed consent For this type of study (retrospective), formal consent is not required.

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