



Development and validation of a host-dependent, PDL1-independent, biomarker to predict 6-month progression-free survival in metastatic non-small cell lung cancer (mNSCLC) patients treated with anti-PD1 immune checkpoint inhibitors (ICI) in the CERTIM Cohort: The ELY study

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ABSTRACT

Background: Immune checkpoint inhibitors (ICI) are dramatically active in a minority of non-small cell lung cancer (NSCLC) patients. We studied here the relationship between patients's metabolism and outcome under ICI.

Methods: Metastatic NSCLC patients underwent a nutritional assessment prior to initiating immunotherapy. Resting energy expenditure (REE) was measured (mREE) using ambulatory indirect calorimetry and compared with the theoretical value (tREE) provided by the Harris and Benedict formula. The primary endpoint was 6-month progression-free survival (PFS). Secondary endpoints included objective response rate (ORR) and disease control rate (DCR) based on investigator review per RECIST v1.1. and overall survival (OS). The association of patient's metabolism with 6-month PFS was first explored in a single-center training cohort to estimate the effect size. The relationship between patient's metabolism and 6-month PFS was then tested in an independent non interventional observational prospective cohort (ELY) of 100 patients recruited in two tertiary university centers.

Findings: In the entire cohort, the ORR was 14% for the hypermetabolic group ($n = 10/74$) vs 38% for the normometabolic group ($n = 26/68$), respectively (estimated difference 25%, 95CI 9–40%, $p = 0.001$). The DCR was 28% for the hypermetabolic group ($n = 21/74$) vs 53% for the normometabolic group ($n = 36/68$), respectively (estimated difference 25%, 95CI 7–42%, $p = 0.005$). In the validation cohort (100 patients, 2 centers), normometabolic patients (defined as $mREE/tREE < 110\%$) had increased 6-month PFS (57% versus 22%; odds ratio: 4.76; IC95 [1.87 – 12.89]; $p < 0.001$) and improved overall survival (HR 2.20; IC95: 1.41–3.44; $p < 0.001$). The positive and negative predictive values of normometabolism to identify non-progressive patients at 6

Abbreviations: BMI, body mass index; CRP, C-reactive protein; DCR, disease control rate; HR, hazard ratio; ICI, immune checkpoint inhibitors; mREE, measured REE; NSCLC, non-small cell lung cancer; PFS, progression-free survival; ORR, objective response rate; OS, overall survival; tREE, theoretical REE by the Harris Benedict formula; REE, resting energy expenditure; VO2, oxygen consumption

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months, were 57% and 78% respectively, sensitivity was 72% and specificity was 66%. In multivariate analysis including PD-L1 tumor status, basal metabolism was an independent predictive factor for 6-month PFS.

Interpretation: Normometabolism is a new independent parameter to identify mNSCLC patients who will benefit from ICI, with both improved tumor response, 6-month PFS, and survival.

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Research in Context

Evidence before this study

This analysis was prompted by the unmet need to identify potential biomarkers related to the host suitable for screening the population that may benefit from immunotherapy. Not all patients with non-small cell lung cancer (NSCLC) benefit from immune checkpoint blockers, and some suffer from notable immune adverse events. At present, tumor PD-L1 expression is the only approved biomarker, albeit imperfect, used in clinical practice for PD- (L)1 blockade in NSCLC. The predictive biomarker role of tumor related factors such as Tumour Mutational Burden remains a challenge in the absence of prospective validation for survival. However, the characteristics of the patients play a central role in driving response to immunotherapy. We searched PubMed on February 15, 2021, for studies reporting on host-dependent biomarker to predict progression-free survival (PFS) in metastatic NSCLC patients treated with anti-PD1 immune checkpoint inhibitors (ICI). We used the search terms « immune checkpoint inhibitors », « predictive biomarker », in combination with « NSCLC » and « host ». The assessment of the prognostic and predictive value of peripheral blood biomarkers in patients treated with ICI has been reported. Different from absolute lymphocyte count which indirectly measures the anti-cancer immune response, absolute neutrophil count, neutrophil-to-lymphocyte ratio (NLR) may reflect cancer-associated inflammation, a key determinant of disease progression in lung cancer. Studies have revealed that gut microbiome could modulate responses to immunotherapy in a vast number of malignancies, including lung cancer. Antibiotics could impair the balance between gut microbiome and the host transiently, as reported by Routy et al., showing shorter PFS in patients with antibiotic-treated NSCLC. Cancer cells consume high levels of glucose resulting in increased lactate production even under aerobic conditions to meet the demands of rapid growth and proliferation. The Warburg effect is catalyzed by the metabolic enzyme LDH. LDH was incorporated together with NLR in the lung immune prognostic index. Hatae et al. investigated levels of plasma metabolites and T cell properties, including energy metabolism markers, in the blood of patients with NSCLC before and after treatment with nivolumab. Combination of biomarkers reflecting host immune activity was quite valuable for responder prediction. Patients with cancer present considerable changes in the homeostasis of energy production and consumption. To date, no study has been published on the role of resting energy expenditure to predict efficacy of anti-PD1 in NSCLC patients.

Added value of this study

We report findings from an evaluation of resting energy expenditure (REE) assessed using indirect calorimetry in the outpatient setting before treatment with ICI. To our knowledge, this study is the first to provide an analysis of REE in ICI-treated metastatic non-small lung cancer patients and to find an association between REE and both 6-month PFS, response rate, and

overall survival. Our study supports other studies that have shown an association between pretreatment performance status and clinical outcome, already suggesting the critical role of the host status. Furthermore, we identified an optimized cutoff point of 110% to separate patients with shorter PFS, lower tumor response and reduced survival. These results were validated in an independent prospective cohort (validation cohort).

Implications of all the available evidence

Reliable tests are needed for clinical practice to predict the anti-tumour activity of ICI in patients with metastatic non-small cell lung cancer. Our results provide evidence that pretreatment REE might serve as an easy-to-use prognostic and predictive parameter to identify patients who could derive clinical benefit from ICI. These findings can inform future studies to evaluate the clinical utility of assessing REE for improved patient management. Moreover, these results open new avenues of therapeutic modulation based on the correction of excessive energy expenditure in patients who are non-responders to ICI. This approach needs a randomized trial to evaluate the impact of the modulation of REE.

1. Introduction

The efficacy of immune checkpoint inhibitors (ICI) targeting programmed cell death protein 1 (PD1) in non-small cell lung cancer (NSCLC) has completely changed the treatment of this disease [1,2]. However, only a minority of patients experience a complete and durable response. Therefore, a priority is to provide reliable biomarkers to identify patients who will respond to ICI. One routinely used biomarker is tumor-related: the over-expression of programmed death ligand 1 (PD-L1) by cancer cells is associated with increased response rate, and improved survival following treatment with ICI [3]. Other parameters associated with outcome are performance status, gut microbiome [4], use of antibiotics [5] and systemic inflammation [6]. Hence, the characteristics of the patients appear of crucial importance in determining the outcome under ICI. Therefore, we aimed to further analyze the host characteristics through the study of the energy expenditure of the patients prior to initiate ICI. Resting energy expenditure (REE) represents the main component of total energy expenditure, well ahead of energy expenditure induced by diet or physical activity. Patients with increased REE by more than 10% are called “hypermetabolic” patients and are at risk of malnutrition and cachexia. Interestingly, increased REE may be detected prior to the occurrence of an alteration of the performance status, in the absence of asthenia, or detectable biological signs of inflammation [7] and appears as an early feature of pre-cachexia [8,9]. We hypothesized that increased energy expenditure would result in a reduced ability of the patient to develop an active immune response following ICI infusion, since the T-cell activation requires metabolic changes to leave the G₀ stage and enter the G₁ stage of the cell cycle, such as enhanced glycolysis, which is a highly ATP-dependent process [10]. We reproducibly found that 50% of NSCLC patients are hypermetabolic, as measured by indirect calorimetry in the outpatient setting [11,12]. The aim of this study was to explore whether the metabolic

status (to have normal versus increased REE) could be associated with the outcome of patients under ICI.

2. Patients and methods

2.1. Study design and population

We conducted a longitudinal, prospective, observational study (ELY) in two tertiary university centers (Cochin hospital and European Georges Pompidou Hospital), which included patients between August 2016 and October 2019 and ended follow-up in April 2020.

We created in July 2015 the CERTIM cohort dedicated to the multidisciplinary assessment and follow-up of cancer patients under ICI. The ELY study includes two cohorts: (i) a training set ($N = 38$), included retrospectively from the CERTIM cohort from July 2015 to July 2016 in Cochin hospital, (ii) a prospective validation set ($N = 106$), included from August 2016 to October 2019 in Cochin and European Georges Pompidou hospital, which aimed to include adult patients with NSCLC initiating nivolumab or pembrolizumab in real-life conditions in Paris. We enrolled consecutive patients who participated in a multidisciplinary risk assessment program in the outpatient unit of the oncology department. This program is proposed to every cancer patient before anticancer therapy initiation, and aims at providing personalized supportive care, summarizing the complexity of the patient, choosing the most adapted treatment and at reducing complications during the course of cancer treatment.

Key eligibility criteria were age 18 years or older, stage IV histologically proven NSCLC and monotherapy with nivolumab or pembrolizumab. Patients were required to have measurable disease per the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1.

Patients were treated with nivolumab, at a dose of 3 mg/kg every 2 weeks, or pembrolizumab, at a dose of 2 mg/kg every 3 weeks.

Exclusion criteria included active malignancy other than NSCLC, ALK or EGFR mutated NSCLC, anticancer therapy or surgery within the past 2 weeks or inability to breathe under the calorimetry. Patients were followed until the date of their death or their last examination. Follow-up period ended on 23th April 2020.

2.2. Ethics

All patients provided written informed consent under a form issued by an institutional review board. This study was approved by the Institutional Review Board (CLEC of Cochin university Hospital University, N°120,518). and conducted according to the declaration of Helsinki. This study is registered with ClinicalTrials.gov, number NCT04879316.

2.3. Data collection and response assessment

2.3.1. Multidisciplinary risk assessment

Patients underwent a multidisciplinary evaluation including consultation with an oncologist and a dietitian. Such an assessment includes subjective and objective parameters such as medical history, weight loss, current dietary intake (including energy and protein balance), physical examination and anthropometric measurements, functional and mental assessment, medications, REE measurement and laboratory values.

REE was determined prior to immunotherapy initiation, under standard resting conditions, i.e. after 12 h of fasting (overnight), between 8 and 9 a.m., in a thermo-neutral environment, by a trained nurse. Prior to commencement of the measurements, patients rested quietly for 15 min, during which time the indirect calorimeter was calibrated and a steady state obtained [13]. Patients were asked to remain awake for the duration of the measurement. For each patient, oxygen consumption (VO_2) was measured for 15 min by indirect

calorimetry using a face mask connected to an oxygen analyzer (Fitmate, COSMED, Italy). The calorimeter was calibrated before each measurement. Measured REE (mREE, kcal/d) was determined from VO_2 using Weir's equation [14] and results were immediately displayed in software attached to the system.

To evaluate the extent of REE alteration compared to healthy individuals, mREE was compared to theoretical REE (tREE), calculated with revised Harris and Benedict equations [15]:

- males: $tREE \text{ (kcal/d)} = 66.5 + 13.75 \times W + 500 \times H - 6.78 \times A$
- females: $tREE \text{ (kcal/d)} = 655 + 9.56 \times W + 185 \times H - 4.68 \times A$
- with W, weight in kilograms; H, height in meters; and A, age in years.

Patients were classified as hypermetabolic or normometabolic function of the ratio of measured REE (indirect calorimetry) to theoretical REE (Harris-Benedict), mREE/tREE ratio, over or equal to 110% or between 90 and 110%, respectively.

Anthropometric measurements included body weight – measured with a medical balance – and height – measured with a stadiometer. Body mass index (BMI) was calculated as weight (kg)/height (m^2). Routine biological tests included serum albumin and plasmatic C-reactive protein (CRP) levels. The Glasgow Prognostic score (GPS) was calculated as follows: the presence of both elevated CRP ($>10 \text{ mg/L}$) and hypoalbuminemia ($<35 \text{ g/L}$) was given a score of 2 and the presence of only 1 parameter or neither of these biochemical abnormalities were given a score of 1 or 0, respectively.

Anonymized clinical data were recorded by local investigators using electronic case report forms (eCRF) in a password-protected secure online portal (Cleanweb).

We counted the number of metastatic sites according to the radiologists' reports of the computed tomography scan and other image examinations taken before the ICI therapy. Best response to immunotherapy, achieved at least once during the course of therapy, was based on Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1.

For each tumor, we performed PD-L1 immunostaining on fresh-cut slides from representative blocks using an anti-PD-L1 antibody (E1L3N, Cell signaling) on Bond automat (Leica) as previously described and validated by the PATTERN French thoracic pathologists group.

2.4. Statistical analyses

Calculations were performed using R statistical software (version 3.6.2., R Stats, epiR, survival and survcomp Package).

Comparisons between groups were performed with Student *t*-test for quantitative variables and with chi-square test for qualitative variables.

The primary endpoint of analysis was 6-month PFS [16]. We first performed a retrospective exploratory study on 38 patients to estimate the effect of hypermetabolism on 6-month PFS. Then we conducted a prospective validation study in two centers to validate our hypothesis. We calculated that we would need to enroll 50 patients in normo- and hypermetabolic groups to show a 30% difference of 6-month PFS (50% vs 20% respectively) with a two-sided 5% significance level and a 90% statistical power.

Logistic regression was used to test the association of clinical and biological variables with 6-month PFS. Variables associated with p -value < 0.1 in univariate analyses and PD-L1 tumor status were included into multivariable models. Interaction tests were performed between patient metabolism and the following variables: age ($<$ or > 70 y), sex, histological subtype (squamous, adenocarcinoma or other), smoking status (never or former/current smoker), ECOG PS (0–1 or ≥ 2), BMI (< 18 , 18–25 or $> 25 \text{ kg/m}^2$), weight loss in the last three

months (< or > 5%), GPS (0 or 1–2), PDL1 status (< or \geq 50%) and treatment type (nivolumab or pembrolizumab). Since there were few missing values (< 10%) for all variables except PD-L1 tumor status, no imputation of missing data was carried out. However, a sensitivity analysis including only complete cases was performed and revealed no significant differences in univariate analyses. Likewise, multivariable analyses were conducted with or without including PD-L1 tumor status.

The calibration of multivariate models was checked using Hosmer-Lemeshow test (adequate calibration with $p > 0.05$ for all models). Finally, Likelihood ratio test was used to check the discrimination performance of nested models ($p < 0.05$ for all models adding basal metabolism).

Secondary endpoints included Objective Response Rate (ORR) and Disease Control Rate (DCR) based on investigator review per RECIST v1.1. and Overall Survival (OS). DCR was defined as complete response (CR) + partial response (PR) + stable disease (SD) per all patients, and ORR as CR + PR per all patients. PFS was calculated from the first day of the immunotherapy administration until progression disease (PD) or death due to any cause. Overall survival (OS) was defined as the time elapsed between evaluation and death or last follow-up visit. For PFS, patients alive without progression at the time of analysis were censored at the initiation of a new therapy or last follow-up. For OS, patients alive at the time of analysis were censored at the last follow-up.

Survival curves were obtained with Kaplan-Meier estimates and compared with log-rank test. Cox proportional hazards regression was used to identify clinical and biological variables associated with OS. The proportional hazards assumption was checked for each model using graphical methods based on Kaplan-Meier curves and the scaled Schoenfeld residuals.

All p -values were two-sided, and the level of significance was set at $p < 0.05$, unless otherwise specified.

Role of the funding source:

Baxter provided a grant for the ELY study. The funder of the study had no direct role in study design, data collection, analysis, or interpretation, or writing of the report, but provided financial support for the recruitment of the research dietician. The corresponding authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

3. Results

We enrolled a total of 144 patients. The clinical cutoff for the primary PFS was on 23th April 2020.

3.1. Patients characteristics

During the study period, 201 patients with NSCLC diagnosis were examined for eligibility and scheduled for nivolumab ($n = 159$) or pembrolizumab ($n = 42$) monotherapy.

One hundred forty-four patients were treated with anti-PD1 and included in the analyses. The main reasons for exclusion were inpatient setting ($n = 28$) and the absence of REE measurement (refusal, non-fasting condition or calorimetry failure) ($n = 24$) (Flow-chart: Supplemental Figure S1).

Patients' characteristics are described in Table 1. Briefly, mean age was 66.8 years (± 9.4), 109 patients (76%) had adenocarcinoma histology, 48 patients had brain metastases, at the time of evaluation. Seventy-three patients (51%) had a performance status of 0 or 1. Forty-three patients (30%) had PD-L1 $> 50\%$ and 45 patients (31%) had unknown PD-L1 status (Nivolumab received FDA approval on March 2015 irrespective of PD-L1 expression). The median number of prior systemic regimens was 1 (range, 0 to 5).

Most individuals were overweight ($n = 54$, 38%) and obese ($n = 25$, 17%). Mean weight loss percentage was 4.9% ($\pm 6.5\%$). Forty-eight

patients (33%) had weight loss $> 5\%$ in the past 6 months. There were no differences in anthropometric, demographic, energy expenditure variables between training and validation cohorts, except for age, albumin and C-reactive Protein levels. Patients in the validation cohort had significantly lower albumin ($p < 0.01$) and higher C-Reactive Protein levels ($p < 0.01$) than in the training set. Patients in the validation cohort were older than in the training set ($p = 0.03$).

A majority of patients were treated on the second line or more ($n = 113$, 78%).

The safety profile was consistent with previous reports of nivolumab and pembrolizumab.

One hundred forty-two patients, including 38 patients in the training set and 104 patients in the validation set -were assessed for Objective Response Rate ORR and Disease Control Rate DCR (Table 1). The objective response rate was 25% ($n = 36$), including 8% complete responses. No significant difference in ORR and DCR was observed between the training and validation set (Table 1).

One hundred thirty-eight patients were assessed for 6-months PFS, with 6 patients (4%) lost to follow-up in the validation set (Table 1). Overall, the 6-month PFS was 33% (95% CI, 27–43). No significant difference in 6-month PFS was observed between the training and validation set ($p = 0.14$) (Table 1).

During the follow-up period, 83 death events (58% of patients) were observed and the median OS was 11.7 months (95% CI, 10.3 to 15.3 months). Twenty-seven patients had ongoing objective responses at data cutoff, and 11 patients continued to receive treatment.

Energy expenditure description mREE in study patients estimated by indirect calorimetry was higher than tREE calculated from revised Harris and Benedict equation (mean 1529 ± 377.3 kcal/d). Mean mREE/tREE ratio was 108.5% ($\pm 19.3\%$). Using cut-off value of 110% in mREE/tREE ratio, a total of 69 (48%) and 75 (52%) patients were classified as normometabolic and hypermetabolic, respectively (Table 1).

Comparisons of baseline characteristics between hypermetabolic (mREE/tREE ratio $> 110\%$) and normometabolic (mREE/tREE ratio $\leq 110\%$) patients is presented in Table 2. Hypermetabolism was associated with lower albumin ($p < 0.001$) and altered Glasgow Prognostic Score (albumine < 35 g/L and CRP > 10 mg/L) ($p = 0.001$). A Glasgow Prognostic Score score of 0 (albumine ≥ 35 g/L and CRP ≤ 10 mg/L) was observed in only 17 (24%) hypermetabolic patients vs 31 (53%) normometabolic patients ($p = 0.001$).

Antitumour Activity according to patients metabolism

In the training cohort (38 pts, one center), hypermetabolic patients defined as measured REE $> 110\%$ of theoretical REE, had worse 6-month PFS (10% versus 39%; odds ratio: 5.46; IC95 0.84–63.21; $p = 0.08$) compared to normometabolic patients. In the validation cohort (100 patients, 2 centers), hypermetabolic patients had significantly worse 6-month PFS (22% versus 57%; odds ratio: 4.76; IC95 1.87–12.89; $p < 0.001$). In the whole cohort, hypermetabolic patients had worse 6-month PFS (18% versus 52%; odds ratio: $p < 0.0001$) compared to normometabolic patients (Table 3).

Interaction tests revealed no significant subgroup difference.

The ORR was 14% for the hypermetabolic group ($n = 10/74$) vs 38% for the normometabolic group ($n = 26/68$), respectively (estimated difference 25%, 95CI 9–40%, $p = 0.001$). The DCR was 28% for the hypermetabolic group ($n = 21/74$) vs 53% for the normometabolic group ($n = 36/68$), respectively (estimated difference 25%, 95CI 7–42%, $p = 0.005$) (Table 3). Among the 32 patients (22%) who received pembrolizumab as first line therapy, because of a high expression of PD-L1 ($\geq 50\%$), it is worthwhile noticing that the response rate was 41%. Among the 16 patients who failed (primary refractory) to pembrolizumab, 10 (62.5%) were hypermetabolic.

In the whole cohort, hypermetabolic patients had significantly worse overall survival: 9.76 months (5.91–11.7) compared to normometabolic patients: 18.99 months (14.39-not reached) ($p < 0.001$) (Table 3) (Fig. 1).

Table 1
Baseline patient characteristics.

	Training set (n = 38)	Validation set (n = 106)	Total (n = 144)	p
Quantitative variables			Mean	
Age (years)	63.9 (9.0)	67.8 (9.3)	66.8 (9.4)	0.03
Qualitative variables			N	
Sex				
Male	22 (58)	70 (66)	92 (64)	0.48
Female	16 (42)	36 (34)	52 (36)	
ECOG- Performance Status				0.36
0–1	23 (61)	50 (47)	73 (51)	
≥ 2	15 (39)	50 (47)	65 (45)	
Unknown	0	6 (6)	6 (4)	
Histological sub-type				0.13
Squamous cell	11 (29)	15 (14)	26 (18)	
Adenocarcinoma	25 (66)	84 (79)	109 (76)	
Large cell neuroendocrine and others	2 (5)	7 (7)	9 (6)	
PD-L1 expression level				0.06
> 50%	5 (25)	38 (48)	43 (30)	
≤ 50%	15 (75)	41 (52)	56 (39)	
Indeterminate, not evaluable or missing			45 (31)	
Treatment				<0.001
Nivolumab	38 (100)	74 (67)	112 (78)	
Pembrolizumab	0	32 (33)	32 (22)	
Smoking Status				0.96
Never smoked	2 (5)	4 (4)	6 (4)	
Former smoker > 1y	4 (12)	11 (11)	15 (10)	
Former smoker < 1y	22 (65)	69 (67)	91 (63)	
Current smoker	6 (18)	19 (18)	25 (17)	
Unknown			7 (5)	
Malnutrition				0.33
No malnutrition	12 (32)	33 (32)	41 (31)	
At risk of malnutrition	17 (46)	32 (31)	49 (34)	
Moderate	6 (16)	28 (27)	34 (24)	
Severe	2 (5)	9 (9)	11 (8)	
Unknown			5 (3)	
Weight loss				1
> 5%	12 (35)	36 (36)	48 (33)	
≤ 5%	22 (65)	65 (64)	87 (61)	
Missing			9 (6)	
BMI (kg/m²)				0.13
< 18	0	5 (5)	5 (3)	
18–25	27 (71)	58 (55)	85 (59)	
> 25	11 (29)	43 (41)	54 (38)	
Albumin (g/L)				0.02
≥ 35	34 (89)	72 (68)	106 (74)	
< 35	4 (11)	34 (32)	38 (26)	
C Reactive Protein (mg/l)				0.09
> 10	18 (49)	62 (67)	80 (56)	
≤ 10	19 (51)	31 (33)	50 (35)	
Missing			14 (10)	
Glasgow Prognostic Score				<0.01
0	18 (49)	30 (32)	48 (33)	
1	16 (43)	30 (32)	46 (32)	
2	3 (8)	33 (35)	36 (25)	
Missing			14 (10)	
Metabolism (mREE/tREE ratio)				1
Normometabolic patients (≤ 110%)	18 (47)	51 (48)	69 (48)	
Hypermetabolic patients (> 110%)	20 (53)	55 (52)	75 (52)	
Best Response				0.24
Confirmed CR, n (%)	5 (13)	7 (7)	12 (8)	
Confirmed PR, n (%)	3 (8)	21 (20)	24 (17)	
SD, n (%)	5 (13)	16 (15)	21 (15)	
PD, n (%)	25 (66)	60 (58)	86 (60)	
Confirmed ORR,% (95% CI)				0.62
0	30 (79%)	76 (73%)	106 (74%)	
1	8 (21%)	28 (27%)	36 (25%)	
Confirmed DCR,% (95% CI)				0.50
0	25 (66%)	60 (58%)	85 (59%)	
1	13 (34%)	44 (42%)	57 (40%)	
6-months PFS (%)	9 (24%)	39 (39%)	48 (33%)	0.14
PFS, median, months (95% CI)	3.38 (1.87–5.19)	3.45 (2.69–7.03)	3.45 (2.69–4.90)	0.44
OS, median, months (95% CI)	11.10 (5.62–14.20)	14.40 (10.32–NR)	11.70 (10.30–15.30)	0.11

Values are expressed in N (%) for qualitative variables and in median (IQR) for quantitative variables. BMI, body mass index; mREE, measured resting energy expenditure; pREE, predicted resting energy expenditure, using Harris and Benedict equations. CR, complete response; PR, partial response; SD, stable disease; ORR, objective response rate (defined as CR + PR); DCR, disease control rate (defined as CR + PR + SD); PFS, Progression-free survival; OS, overall survival; CI: confidence interval.

Table 2
Associations between basal metabolism (mREE/tREE ratio) and clinical and biological data.

Variables		Basal metabolism		p-value
		Hypermetabolic patients N = 75	Normometabolic patients N = 69	
Quantitatives variables				
Age, mean, years		66.7 (9.7)	66.8 (9.1)	0.99
Qualitatives variables				
Sex				
	Male	51 (68%)	41 (59%)	0.37
	Female	24 (32%)	28 (41%)	
ECOG-PS				
	0–1	32 (46%)	41 (60%)	0.12
	≥ 2	38 (54%)	27 (40%)	
Smoking status				
	Never	3 (4%)	3 (5%)	0.16
	Former smoker > 1y	12 (17%)	3 (5%)	
	Former smoker < 1y	44 (61%)	47 (72%)	
	Current smoker	13 (18%)	12 (18%)	
Histology				
	Squamous cell	18 (24%)	8 (12%)	0.14
	Non-squamous cell	52 (69%)	57 (83%)	
	Large cell neuroendocrine and others	5 (7%)	4 (6%)	
Malnutrition				
	No malnutrition	19 (26%)	26 (39%)	0.06
	At risk of malnutrition	23 (32%)	26 (39%)	
	Moderate	21 (29%)	13 (19%)	
	Severe	9 (12%)	2 (3%)	
Weight loss				
	> 5%	30 (42%)	18 (29%)	0.16
	≤ 5%	42 (58%)	45 (71%)	
Albumin (g/L)				
	≥ 35	47 (63%)	59 (86%)	<0.01
	< 35	28 (37%)	10 (14%)	
C Reactive Protein (mg/l)				
	> 10	53 (74%)	27 (47%)	<0.01
	≤ 10	19 (26%)	31 (53%)	
Glasgow Prognostic Score (GPS)				
	0	17 (24%)	31 (53%)	0.001
	1	29 (40%)	17 (29%)	
	2	26 (36%)	10 (17%)	
BMI (kg/m ²)				
	<18	4 (5%)	1 (1%)	0.12
	18–25	48 (64%)	37 (54%)	
	> 25	23 (31%)	31 (45%)	

3.2. Patient metabolism is independently associated with anti-PD1 efficacy

Patients metabolism was then explored as associated with the 6-month PFS under anti-PD1.

Table 3
Efficacy of Nivolumab and Pembrolizumab monotherapy in hypermetabolic patients and normometabolic patients.

	Hypermetabolic patients N = 74	Normometabolic patients N = 68	P
Best Response			< 0.01
Confirmed CR, n (%)	3 (4%)	9 (13%)	
Confirmed PR, n (%)	7 (9%)	17 (25%)	
SD, n (%)	11 (15%)	10 (15%)	
PD, n (%)	53 (72%)	32 (47%)	
Confirmed ORR,% (95% CI)			< 0.01
0	64 (86%)	42 (62%)	
1	10 (14%)	26 (38%)	
Confirmed DCR,% (95% CI)			< 0.01
0	53 (72%)	32 (47%)	
1	21 (28%)	36 (53%)	
6-months PFS, months (%)	13 (18%)	35 (52%)	< 0.0001
PFS, median, months	2.05 (1.81–3.19)	7.03 (4.17–10.74)	< 0.0001
OS, median, months (95% CI)	9.76 (5.91–11.7)	18.99 (14.39 - NR)	< 0.001

CR, complete response; PR, partial response; SD, stable disease; ORR, objective response rate (defined as CR + PR); DCR, disease control rate (defined as CR + PR + SD). PFS, Progression-free survival; OS, Overall survival; CI: confidence interval.

In univariate analysis of 6-months PFS, mGPS ($p = 0.03$), increased weight loss ($p = 0.07$) and basal metabolism hyper vs normometabolism ($p < 0.001$) were identified as adverse prognostic factors in the validation cohort (Table 4).

The positive and negative predictive values of normometabolism to identify non progressive patients at 6 months, were 57% and 78% respectively, sensitivity was 72%, specificity was 66%.

In multivariable analysis of 6-month PFS including GPS 1–2 vs 0, basal metabolism (hyper vs normometabolism) and PD-L1 status ($> 50\%$ vs $\leq 50\%$), basal metabolism (hyper vs normometabolism) was an independent factor (OR 3.08; IC95 1.02–9.34; $p = 0.047$).

4. Discussion

This prospective bicentric study is the first report of a relationship between the resting energy expenditure of cancer patients and their sensitivity to ICI. Indeed, patients with increased REE, so called hypermetabolic patients, were less sensitive to ICI. As we hypothesized, the population of normometabolic patients had a 30% improvement in 6-month PFS. Moreover, both tumor response and overall survival were significantly lower in hypermetabolic patients.

PD-L1 tumor status represents the most common biomarker of PD1 efficacy. However, a minority of tumours without PDL1 expression may respond to ICI and some patients do not experience tumor response despite high PDL1 expression. tumor signatures based on transcriptomic analysis may improve the identification of potential responders [8,17]. Here, we identified a host-dependent, PD-L1 independent parameter associated with outcome under ICI.

This report is the first study including a measurement of hypermetabolism in NSCLC patients treated with immunotherapy. The diagnosis of hypermetabolism can be obtained in routine practice in

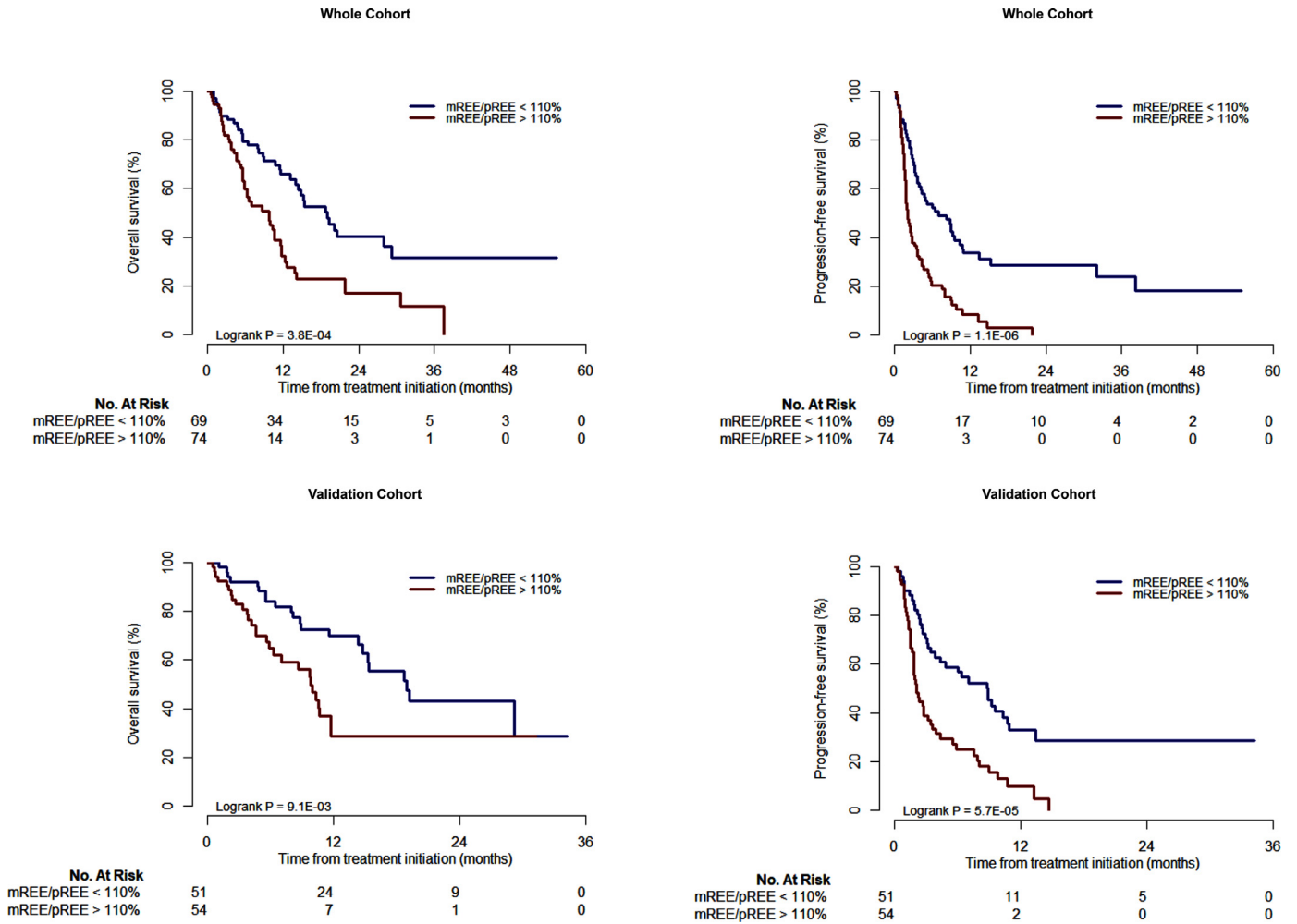


Fig. 1. Overall survival and Progression-free survival according to metabolism status.

the outpatient setting using indirect calorimetry. The result is immediately available for the physician. A vast array of phenomena can increase resting energy expenditure in cancer patients. The energetic demand of the tumor itself, changes in inflammation, body composition and brown adipose tissue activation will result in increased REE. We confirmed in this study the high prevalence of hypermetabolism-around 50% of patients- previously reported by our group in NSCLC patients [12]. Since an accurate assessment of energy needs of the patient is a cornerstone of adequate nutritional therapy, this result is already important to optimize the global treatment of the patient by adjusting energy intakes to measured energy needs [18,19]. Hypermetabolism was associated in this study with lower albumin and altered Glasgow Prognostic Score (albumine < 35 g/L and CRP > 10 mg/L). As a consequence of systemic inflammation concomitant with a tumor, findings report an association between inflammation and REE [20,21] and weight loss [21,22]. Although hypermetabolism is part of precachexia and cachexia syndroms, hypermetabolism may be observed while the patient has no evidence of weight loss nor presence of systemic inflammation [23,24].

Several limitations of these results may be pointed out: the study is not randomized and therefore we cannot discriminate between the relative predictive and the relative prognostic value of hypermetabolism. The prognostic influence of REE and its predictive capacity to identify patients sensitive to ICI therapy are not visible in a dataset, where all patients have been treated with ICI. However, we can notice that hypermetabolism is not only correlated with progression-free survival and overall survival, the REE is also associated with

reduced tumor response rate, suggesting a direct link between whole-body energy and the possibility of a pharmacodynamic effect of ICI. Therefore, REE has both a predictive and prognostic value, but their relative weight has to be determined, through randomized study.

Indirect calorimetry is well known in clinical nutrition but not used in routine practice in oncology. External validation of these results is warranted in a larger number of participating cancer centers and in a larger cohort.

To generate an immune response is a considerable bioenergetic challenge [10]. The T-cells metabolically switch between resting and proliferative states, actively acquire metabolic substrates from their environment to meet these energy demands and respond appropriately to tumours [25]. Lymphocytes that do not receive these signals fail to increase their metabolism to meet the higher bioenergetic demands of cell growth and are either deleted or rendered unresponsive to mitogenic signals [26].

Since hypermetabolism indicates that increased energy is spent by the patient in resting conditions, to maintain vital homeostasis, we hypothesized that hypermetabolic patients might have less remaining energy to fuel lymphocytes and experience lymphocyte-mediated tumor response [27,28]. The results corroborate this hypothesis and now invite to analyze in-depth the mechanisms of hypermetabolism in cancer patients. The final goal would be to provide corrective interventions to reverse hypermetabolism to normometabolism and thereby to improve the responsiveness to immunotherapies. We are presently designing a multicentric

Table 4
Predictive factors of antiPD1 efficacy.

	Univariate analyses						Multivariate analyses					
	Training set (N = 38)			Validation set (N = 100)			Not including PD-L1 (N = 117)			Including PD-L1 (N = 79)		
	OR	95 CI	p	OR	95 CI	p	OR	95CI	p	OR	95CI	p
Age												
/y increase	1.01	0.93–1.11	0.75	1.01	0.97–1.06	0.54						
Sex												
Male	1			1								
Female	1.63	0.34–7.79	0.54	0.78	0.34–1.80	0.56						
ECOG- Performance Status												
0–1	1			1								
≥ 2	0.71	0.15–3.41	0.67	1.02	0.45–2.30	0.97						
Histological sub-type												
Squamous cell	1			1								
Adenocarcinoma	1.42	0.24–8.48	0.70	2.02	0.59–6.90	0.26						
Large cell neuroendocrine and others	4.50	0.19–106.83	0.35	1.1	0.15–8.13	0.93						
PDL1 expression level												
≤ 50%	1			1								
> 50%	0.69	0.06–8.15	0.77	2.07	0.79–5.43	0.14			2.49	0.86–7.16	0.092	
Smoking Status												
Never smoked	1			1								
Former or current smoker	0	0-Inf	0.99	1.61	0.22–11.95	0.64						
Weight loss												
≤ 5%	1			1								
> 5%	0.53	0.09–3.18	0.49	0.43	0.17–1.06	0.07	0.57	0.23–1.41	0.22	0.49	0.16–1.49	0.21
Glasgow Prognostic Score												
0	1			1								
1–2	0.18	0.03–1.06	0.06	0.37	0.15–0.94	0.03	0.46	0.19–1.08	0.07	0.41	0.13–1.27	0.12
Metabolism (mREE/pREE ratio)												
Hypermetabolic patients (> 110%)	1			1								
Normometabolic patients (≤ 110%)	5.73	1.00–32.67	0.049	4.85	2.02–11.63	< 0.001	4.25	1.80–10.01	< 0.001	3.08	1.02–9.34	0.047

randomized trial in hypermetabolic patients comparing continuation of immunotherapy versus continuation of immunotherapy combined with modulation of hypermetabolism.

5. Contributors

F. Goldwasser had the idea for and designed the study and provided financial support. PBR, JAr, CG, SDP, CVV, NR, EF, DD, AML and FGo were involved in acquisition of the data. PBR summarized the data. PBR, JAr, FGo, AJ and GU were involved in data interpretation. PBR, FGo drafted the manuscript. JAr, SDP, EF, AJ, MA, ACP, FGi, NR, DD, AML, JAl, MW, JPD and critically revised the manuscript. All authors read and approved the final version of the manuscript. PBR, JAr, FGo and AJ have verified the underlying data presented in this study.

Data sharing

Data sharing requests will be considered by the co-authors upon written request to the corresponding author. Deidentified participant data or other prespecified data will be available subject to a written proposal and a signed data sharing agreement.

Declaration of Competing Interest

Dr. Boudou-Rouquette reports grants from BAXTER, during the conduct of the study; personal fees from Takeda, personal fees from Pharmamar, outside the submitted work. Pr. Goldwasser reports grants and personal fees from BAXTER, personal fees from FRESENIUS KABI, personal fees from NUTRICIA, outside the submitted work. Pr. WISLEZ reports personal fees from Boeringher Ingelheim, personal fees and non-financial support from ROCHE, personal fees and non-financial support from MSD, personal fees from BMS, personal fees from Astra Zeneca, personal fees from Amgen, personal fees from Janssen, outside the submitted work. Dr. Fabre reports grants from BMS, board membership from ROCHE, Astra Zeneca, and payment for

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Supplementary materials

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