Circulating Fatty Acid Profile as a Biomarker for Immunotherapy in Advanced Non-Small Cell Lung Cancer

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Abstract

We investigated the influence of baseline circulating fatty acids (FAs) on outcome upon immunotherapy in 112 advanced non-small cell lung cancer (NSCLC) patients. We identified a positive association with some esterified middle chain (C18:0) and unsaturated (C16:1) FAs, a negative association with an esterified saturated FA (C16:0). These results suggest an influence of FA metabolism on immunotherapy activity in NSCLC.

Introduction: Lipid metabolism impacts immune cell differentiation, activation, and functions, modulating inflammatory mediators, energy homeostasis, and cell membrane composition. Despite preclinical evidence, data in humans lack concerning tumors and immunotherapy (IO). We aimed at investigating the correlations between circulating lipids and the outcome of non-small cell lung cancer (NSCLC) patients treated with IO. **Materials and Methods:** We identified all patients with advanced NSCLC treated with IO at our Institution with available baseline plasma samples. Fatty acids (FAs) were analyzed through gas chromatography. Survival curves were estimated by the Kaplan-Meier method. Cox multivariate models were constructed through a stepwise procedure, with entry and exit *P* value set at .2. **Results:** We identified 112 patients, mostly with performance status 1 (65.2%) and PD-L1≥1% (75.3%). Median progression-free survival (PFS) and overall survival (OS) were 2.8 and 11.0 months, respectively. Multivariable model for survival identified a positive association of circulating free (FFA) C16:0 (*P* .005) and esterified (EFA) C16:1 (*P* .030) with PFS, and a positive association of EFA C16:1 (*P* .001) and EFA C18:0 (*P* .020) with OS. EFA C16:0 was negatively associated with PFS (*P* .008). **Conclusion:** FFA C16:0 and FAs derived from its unsaturation (EFA C16:1) and elongation (EFA C18:0) are associated with a better outcome in NSCLC patients treated with IO. It is conceivable that the ratio among those FAs may modify membrane fluidity and receptor activity, influencing IO efficacy. These data pave the way for the investigation of lipid-modulating strategies in association with IO in NSCLC.

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Introduction

Immunotherapy (IO) has profoundly changed the treatment paradigm of non-small cell lung cancer (NSCLC).[1] Several trials have shown its efficacy in the first and subsequent therapy lines for the advanced disease, and promising data are also emerging for the initial stages.[2,3] Nonetheless, only a minority of patients gain substantial benefit from IO, and biological bases of response are still unclear.[4,5]

Recent preclinical data have underlined the impact of lipid metabolism in regulating immune functions.[6,7] These works suggest a potential role of lipid mediators in modulating the individual sensitivity to IO.[8] Indeed, tumor tissues are characterized by an aberrant activation of de novo lipogenesis due to an overexpression of fatty acid synthase, ATP citrate lyase, and acetyl-CoA carboxylase, which is correlated to unfavorable cancer outcomes.[9,10] Lipogenesis upregulation favors cancer cell proliferation by a continuous substrate supply for cellular membrane generation and bio-energy production.[11] Clinical lipidomic studies are able to discriminate malignant from normal tissue and reflect response and/or resistance to anticancer treatments.[12] On the other side, lipid metabolism affects the differentiation, proliferation, and activation of some immune cell subsets, including lymphocytes and macrophages, potentially contributing to the balance of the cancer-immune cycle. Cholesterol and fatty acids (FAs) are crucial in the functional regulation of tumor, but also of both innate and adaptive immunity. For example, the inhibition of cholesterol esterification enhances T-cell receptor clustering and formation of the immunological synapse[13], therefore potentiating the anti-tumor activity of CD8 T-lymphocytes.[10] Similarly, FA oxidation is the preferential metabolic route occurring in T-cells switching from a naïve to a memory phenotype[14,15], and FAs directly influence membrane composition and PD-L1 expression on cancer cells.[16] The myeloid immune compartment displays high sensitivity to lipid mediators, too.[17] The shift of tumor-associated macrophages towards a pro-tumor M2 phenotype and pathways involved in emergency granulopoiesis (RORγ/RORC1) are both influenced by lipid metabolism.[18-20] FAs regulate intra-tumor myeloid-derived suppressor cells activity through the specific receptor FATP2, which is involved in the uptake of arachidonic acid and the synthesis of prostaglandin E2.[21,22] Genetic and environmental factors, but also lifestyle choices including physical activity and diet habits, influence lipid metabolism and phenotype, and play a significant role in cancer onset, progression, and treatment response.[23,24] Indeed, diet-related obesity is associated to many diseases including cancer and represents a higher risk for recurrence, comorbidity, and therapy resistance.[25] Really, it is very difficult to assess the role of individual dietary component on the risk of cancer, given their shared common sources and potential synergistic or counteractive effect on the health outcomes.[26]

However, despite consistent preclinical evidence on the multifaceted interplay between tumor, immunity, and lipid metabolism, clinical evidence in cancer patients is currently missing. This study aimed to investigate the impact of individual lipid profile on survival in a cohort of advanced NSCLC patients treated with IO.

Materials and Methods

We reviewed all cases of advanced NSCLC patients treated with IO at our Institution from July 2015 to January 2020. All these patients were enrolled in the prospective trial APOLLO (INT22/15), which needed blood sample collection at IO baseline. Eligibility required a performance status (PS) according to Eastern Cooperative Oncology Group (ECOG) of 0 or 1, a diagnosis of NSCLC at stage IV or III not amenable of local therapies according to American Joint Committee on Cancer Staging (8th edition), and the availability of frozen plasma samples collected within 1 month since the first IO administration. This single Institution observational study was approved by Institutional Review Board in March 2015. All involved patients signed written informed consent for the usage of data and biological samples for research purposes.

Clinical and biological data were retrieved from the Institutional database. Body mass index (BMI), registered at the beginning of IO, was calculated as weight (kg)/height (m)^2. Standard cut-offs for the definition of BMI classes were applied. Disease response was assessed through Radiologic Evaluation Criteria in Solid Tumors (RECIST) version 1.1. Toxicity was graded according to Common Terminology Criteria for Adverse Events (CTCAE) v5.0. The adverse events which occurred before the advent of CTCAE v5.0 (2017) were retrospectively reviewed by the investigators and graded according to the most recent classification. PD-L1 expression was determined by the local standard test; as the assessment method varied during the enrollment period, PD-L1 could be classified only as positive and/or expressed or negative and/or absent without further stratification for the expression level. Neutrophil/lymphocyte ratio (NLR) was derived from the standard blood cell count.

Methods of Biological Analyses

Whole blood samples were collected in 10 mL Vacutainer tubes with spray-coated K2EDTA and stored at room temperature. Plasma was separated by 2 centrifugation steps at 1,258 x g and 4°C for 10 minutes, then held at -80° until use. Starting from 200 µl of plasma, total cholesterol, LDL HDL, and triglycerides were analyzed using the Cobas Roche automated clinical chemistry analyzer (Roche Diagnostics), following the standard clinical procedures.

Plasma esterified FAs (EFAs), representing the components included in lipoproteins, were analyzed as methylesters, while the circulating albumin-bound components were analyzed as free FAs (FFAs). The main dosed FAs (as EFA and FFA) were C16:0 (palmitic acid), C16:1 (palmitoleic acid), C18:2 (linoleic acid), C18:3α (α-linolenic acid), C20:4 (arachidonic acid), C20:5 (eicosapentenoic acid), C22:6 (docosahexenoic acid).

Plasma EFAs were obtained by direct derivatization of an aliquot of plasma with sodium methoxide in methanol 3.33% (w/v). EFAs were rapidly extracted with hexane, rapidly dried, and was quantified by gas chromatography equipped with a flame ionization detector.[27] FFAs were extracted with 3 different chloroform and/or methanol mixtures (1:1, 1:2 and 2:1, v/v) containing 50 μM 2,6-bis(1,1-dimethylethyl)-4-methylphenol (BHT) according to Folch, with minor modifications, and their distribution and content were assessed by gas chromatography with flame ionization detector.[28]

Table 1 Patient, Tumor, and Treatment Characteristics

Characteristics	N = 112
Gender	
Male	76 (67.9)
Female	36 (32.1)
Age	
Median	68.0
Q1-Q3	60.5-73.5
Smoking status	
Former/current smoker	96 (85.7)
Never smoker	16 (14.3)
BMI	
<18.5	3 (2.9)
18.5-24.9	53 (51.0)
25.0-29.9	37 (35.6)
30.0-35.9	11 (10.5)
Not available	8
ECOG PS	
0	39 (34.8)
1	73 (65.2)
NLR	
<5	74 (69.2)
≥5	33 (30.8)
Missing	5
Disease stage	
IIIB	1 (0.9)
IIIC	1 (0.9)
IV	110 (98.2)
Histology	
Non-squamous NSCLC	90 (80.4)
Squamous NSCLC	22 (19.6)
Sites of distant disease	
1	38 (33.9)
≥2	74 (66.1)
PD-L1 status	
Positive	67 (75.3)
Negative	22 (24.7)
Not available	23
IO agent	
Anti-PD1	101 (90.2)
Pembrolizumab	*50 (44.6)*
Nivolumab	*51 (45.5)*
Anti-PDL1	8 (7.1)
Atezolizumab	*7 (6.3)*
Durvalumab	*1 (0.9)*
Anti-CTLA4/combined IO	3 (2.7)
Durvalumab+tremelimumab	*2 (1.8)*
Tremelimumab	*1 (0.9)*
Line of IO	
First	45 (40.2)
Second	52 (46.4)
Third or more	15 (13.4)
IO status	
Ongoing	27 (24.1)
Discontinued	85 (75.9)

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Table 1 (*continued*)

Characteristics	N = 112
PD	*66 (58.9)*
Toxicity	*10 (8.9)*
Clinical decision/conclusion	*5 (4.5)*
Lost at follow-up	*4*

Abbreviations: BMI = body mass index; ECOG PS = Eastern Cooperative Oncology Group performance status; IO = immunotherapy; N = number; NLR = neutrophil/lymphocyte ratio; NSCLC = non-small cell lung cancer; PD = disease progression; Q1-Q3 = inter-quartile range.

The separation was achieved as follows: capillary Nukol, 15 m x 0.53 mm I.D, 0.5 µm; carrier gas, helium; injector temperature, 250°C; detector temperature, 250°C; oven temperature was controlled at 110°C for 2 minute and then increased at a rate of 8°C minute−1 to 220°C. A standard mixture containing all EFA methylesters (Avanti Polar Lipids Inc, Alabaster, USA) and FFAs (Avanti Polar Lipids Inc, Alabaster, USA) was injected for the calibration curve. The identities of plasma FA peaks were determined by comparing their relative retention times with those of well-known EFA and FFA standards. Before manipulation, TG C17:0 and C17:0 were added as internal standards. EFAs and FFAs were quantified using the chromatographic peak area according to the internal standard (IS) method.

Statistical Analysis

Continuous variables were described as the number of observations, median, inter-quartile range (Q1-Q3), and missing values. For categorical variables, the frequency and percentage of subjects in each category were reported. Overall response rate (ORR) was defined as the rate of patients achieving a complete (CR) or partial (PR) response during treatment. For ORR, the 95% confidence interval (CI) was computed through exact binomial methods.

Progression-free survival (PFS) was calculated as the time from the beginning of IO to disease progression, or death for any cause. For patients continuing IO beyond PD, the date of the first PD was considered for PFS. Overall survival (OS) was calculated as the time from the beginning of IO to death for any cause. Time-to-event variables were estimated through the Kaplan-Meier method. For the purposes of the survival analyses, FAs were considered as continuous variables to optimize the statistical power and avoid potential biases related to dichotomization.

For PFS and OS, a Cox regression model was constructed by stepwise selection with entry and exit *P* value set at .2. The most important prognostic clinical and demographic characteristics (line of IO, histology, ECOG PS) were included in the final multivariable model without the process of selection. To avoid overfitting problems, 1 covariate for 10 events could be included in the multivariable models[29]; therefore, 9 variables for PFS and 7 variables for OS were analyzed. PD-L1 was not considered for the multivariable models due to the high number of missing data and its high correlation with the IO line, leading to multicollinearity. The survival models were repeated considering lipid modulating agents as a covariate to exclude a critical influence of pharmacologic intervention on results. To include all the patients in the multivariable models, some missing values were imputed using medians. Multivariable models were constructed by stepwise selection with a .2 entry and .05 exit *P* value.

The correlation between lipid and main clinical variables was analyzed through the Pearson correlation coefficient (for continuous variables) and a t-test (for dichotomous variables).

A P value $< .05$ was considered statistically significant. All the analyses were performed using SAS (Statistical Analysis System version 9.4, SAS Institute, Cary, NC, USA).

Results

Patient and Tumor Characteristics

A total of 112 patients were included (Supplementary figure 1).

Seventy-six (67.9%) were males and 36 (32.1%) females. The median age was 66.6 years (Q1-Q3: 60.5-73.5). Most patients (96/112, 85.7%) were former or current smokers at the beginning of IO. The median basal BMI was 24.6 kg/m^2 (Q1-Q3: 22.1-27.8). According to standard classification, 3 patients (2.9%) were underweight, 53 (51.0%) had a normal BMI, 37 (35.6%) were overweight, 11 (10.6%) were obese. Eighteen patients (16.1%) received treatment with a lipid-modulating agent (a statin in 13 cases, a fibrate in 2 cases, both drugs in 1 case). At the first IO administration, ECOG PS was 0 in 39 patients (34.8%), 1 in 73 patients (65.2%). The median value of NLR at baseline was 3.8 (Q1-Q3: 2.1-5.6); NLR was below the conventional cut-off of 5 in 74 cases (69.2%). The disease stage was IIIB in 1 case, IIIC in 1 case, IV in the remaining 110 cases. Tumor histology was non-squamous in 90 cases (80.4%), squamous in 22 cases (19.6%). Most patients (74/112, 66.1%) had 2 or more distant disease sites at the beginning of IO. PD-L1 was defined as positive in 67 patients (75.3%) and negative in 22 patients (24.7%); PD-L1 status was unavailable in 23 cases. No patients had driver genetic alterations (EGFR mutation, ALK or ROS-1 rearrangement). Patient and tumor characteristics are detailed in Table 1.

IO Treatment Characteristics

One hundred-one patients (90.2%) received an anti-PD1 agent, 8 (7.1%) an anti-PDL1 agent, 3 (2.7%) an anti-CTLA4 or an IO-IO combination. IO was administered as the first line in 45 cases (40.2%), as the second line in 52 cases (46.4%), as the third or more advanced line in 15 cases (13.4%). The reason for IO discontinuation was mainly disease progression (66/112, 58.9%), followed by toxicity (10/112, 8.9%) and clinical decision and/or regular end of treatment (5/112, 4.5%); 4 patients were lost at follow-up. Treatment was ongoing at the time of database lock in 27 cases (24.1%). Toxicity graded 2 or more according to CTCAE was registered in 40 patients during IO; 14 patients experienced severe (G3-4) toxicity, mostly pneumonia and transaminitis (3 cases each). IO treatment details are summarized in Table 1.

Table 2 Descriptive Statistics of Standard Laboratory Variables

Variable	N = 112 (%)
Total cholesterol (mg/dL)	
Mean (SD)	172.8 (37.3)
Median (Q1-Q3)	175.0 (148.0-199.0)
Min-Max	86.0-279.0
Missing	1
HDL cholesterol (mg/dL)	
Mean (SD)	46.4 (15.0)
Median (Q1-Q3)	43.0 (36.0-53.0)
Min-Max	12.0-198.0
Missing	1
LDL cholesterol (mg/dL)	
Mean (SD)	100.8 (30.0)
Median (Q1-Q3)	102.0 (82.0-121.0)
Min-Max	12.0-198.0
Missing	1
Triglycerides (mg/dL)	
Mean (SD)	128.0 (60.9)
Median (Q1-Q3)	117.0 (86.0-153.0)
Min-Max	39.0-455.0
Missing	1

Abbreviations: HDL = high-density cholesterol; LDL = low-density cholesterol; N = number; Q1-Q3 = inter-quartile range; SD = standard deviation.

Results of Laboratory Analyses

Descriptive statistics of cholesterol (total, LDL, HDL) and triglycerides are reported in Table 2. Descriptive statistics of FAs are reported in Table 3.

Disease Outcome Upon IO

After a median follow-up of 25.4 months, 62 patients (55.4%) progressed and 74 (66.1%) died. Median PFS was 2.8 months, median OS 11.0 months (Figure 1).

ORR was 19.6%, disease control rate (DCR) 54.5%. Thirty-one patients (27.7%) continued IO beyond PD, obtaining at least a SD as the best response in 17 cases.

Multivariate Models for Survival With Stepwise Selection of Variables

High levels of EFA C16:0 (HR 1.16, 95% CI 1.04-1.39; *P* .008) showed a negative impact on PFS; high levels of EFA C16:1 (HR 0.68, 95% CI 0.48-0.96; *P* .030) and FFA C16:0 (HR 0.91, 95% CI 0.85-0.97; *P* .005) showed a positive impact on PFS. Further clinical variables selected in the models were smoking status (HR 1.72, 95% CI 0.92-3.24; *P* 0.092) and NLR (HR 1.15, 95% CI 1.09-1.21; *P* < .001).

Regarding OS, adjusted for the same variables, a positive prognostic role of older age (HR 0.97, 95% CI 0.95-1.00; *P* .047), high levels of EFA C16:1 (HR 0.97, 95% CI 0.95-0.99; *P* .001) and EFA C18:0 (HR 0.73, 95% CI 0.55-0.95; *P* .020) were detected. The negative impact of high NLR was also confirmed on OS (HR 1.19, 95% CI 1.12-1.27; *P* < .001).

The Cox regression models for PFS and OS with the stepwise selection approach are detailed in Table 4.

The results of the Cox models for survival did not change considering the use of lipid-modulating agents at baseline as a covariate (Supplementary Table 1).

Figure 2 shapes the prognostic role of main lipid variables through the adjusted 1 year survival probability estimate in the overall population.

Correlation Analysis Between Lipid and Clinical Variables

Considering lipid variables (FAs, cholesterol, and triglycerides), a strong positive correlation emerged between most EFAs and triglycerides (eg, correlation index 0.823, *P* < .001 for EFA C16:0; correlation index 0.817, *P* < .001 for EFA C18:1). A positive but weaker correlation was confirmed between most FFAs and triglycerides (eg, correlation index 0.306, *P* .001 for FFA C16:0; correlation index 0.348, *P* < .001 for FFA C18:0). Also, cholesterol showed a moderate positive correlation with both EFAs (eg, correlation index 0.490, *P* < .001 for EFA C18:0; correlation index 0.571, *P* < .001 for EFA C18:2), and FFAs (correlation index 0.435, *P* < .001 for FFA C18:0 and FFA C18:2).

Performing the analysis with main clinical variables, absent or very weak correlations emerged between FAs and age, BMI, histology, number of metastatic sites, and smoking status. On the contrary, several significant correlations were evidenced between gender and some EFAs (eg, t-value 3.77 -mean 102.5 vs. 88.1 for female and male, respectively-, *P* .001 for EFA C18:0) and FFAs (eg, t-value 2.67 -mean 159.0 vs. 141.5 for female and male, respectively-, *P* .009 for FFA C16:0; t-value 3.31 -mean 52.4 vs. 46.7 for female and male, respectively-, *P* .001 for FFA C18:0; t-value 2.95 -mean 114.6 vs. 97.0 for female and male, respectively-, *P* .005 for FFA C18:1; t-value 3.56 -mean 89.8 vs. 78.2 for female and male, respectively, *P* .001 for FFA C18:2).

The full results of the correlation analyses are reported in Supplementary table 2.

Discussion

Our study showed for the first time a positive correlation between 3 FAs (FFA C16:0, EFA C16:1, EFA C18:0) and outcome in a cohort of advanced NSCLC patients treated with IO.

IO efficacy relies on a multifaceted interplay among tumor biology, immune system, and drug activity. Individual metabolic profile plays a role in this process, modulating cancer biology and immune cell functioning.[30,31] However, no data have been obtained about the prognostic relevance of baseline lipid asset in cancer patients treated with IO. We investigated this topic in our cohort of advanced NSCLC. To this end, we proceeded in dosing cholesterol, triglycerides, and FAs in plasma samples obtained before the first IO administration.

FA concentration has been expressed as both absolute and relative values. This is a recommended approach to report FA analyses, as the 2 measurements' information is complimentary. Relative concentration allows to gather the complete FA profile of each sample, is more stable than absolute concentration, and is normally distributed. Absolute concentration is appropriate to estimate the

Figure 1 Kaplan-Meier curves of PFS (left) and OS (right).

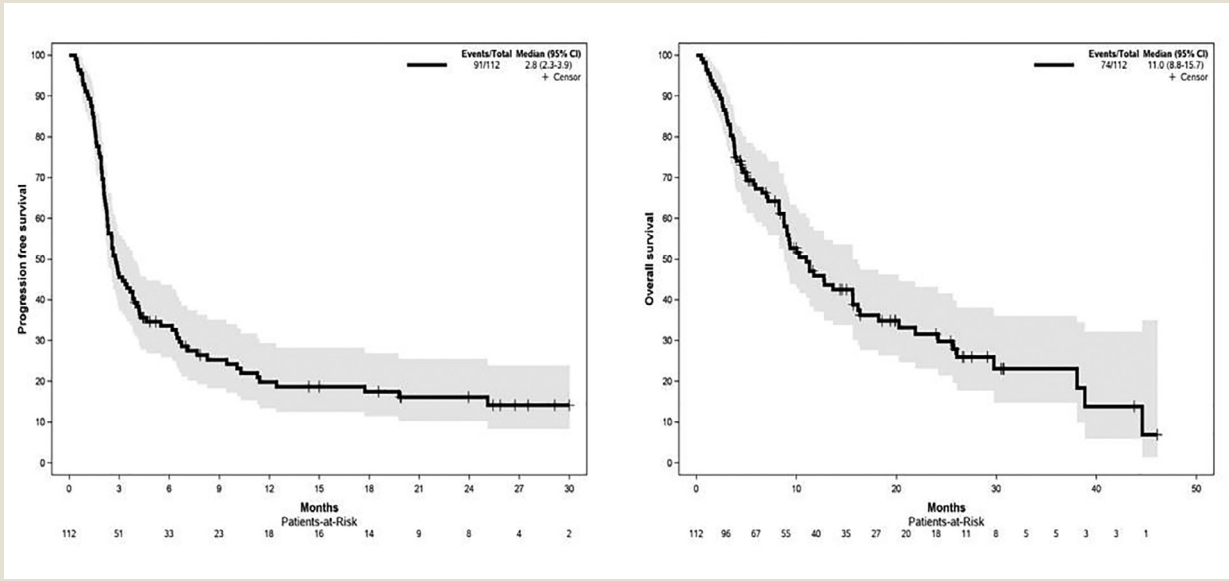

Figure 2 Three-months progression-free probability and 1 year survival probability estimates according to main prognostic lipid variables (adjusted for line of IO, histology, PS, smoking status, gender, and age).

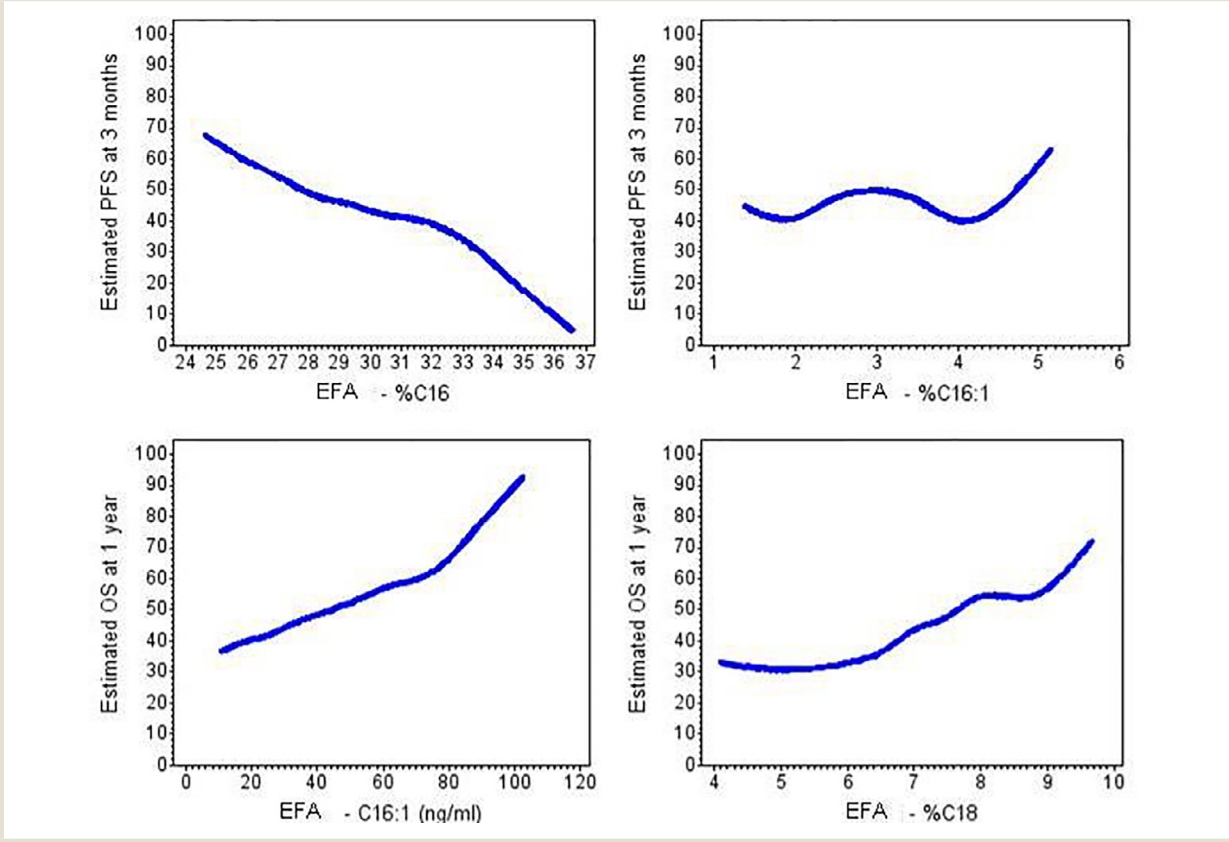

Table 3 Descriptive Statistics of FA Variables

Variable	N = 112 (%)	
EFA	**Relative concentration(%)**	**Absolute concentration (μg/mL)**
C16:0 (palmitic acid)		
Mean (SD)	29.3 (2.4)	372.3 (100.5)
Median (Q1-Q3)	29.1 (27.6-30.7)	360.3 (300.5-412.2)
Min-Max	24.6-36.6	196.5-780.6
Missing	0	0
C16:1 (palmitoleic acid)		
Mean (SD)	2.7 (0.8)	35.5 (16.4)
Median (Q1-Q3)	2.8 (2.2-3.2)	32.9 (24.5-42.5)
Min-Max	1.4-5.1	10.8-102.4
Missing	0	0
C18:2 (linoleic acid)		
Mean (SD)	22.8 (3.4)	286.6 (68.9)
Median (Q1-Q3)	22.4 (20.2-25.1)	280.4 (241.9-330.9)
Min-Max	15.1-31.7	100.4-525.5
Missing	0	0
C18:3α (α-linolenic acid)		
Mean (SD)	0.3 (0.3)	4.1 (3.1)
Median (Q1-Q3)	0.3 (0.3-0.3)	3.6 (3.0-4.2)
Min-Max	0.2-2.9	2.0-33.3
Missing	0	0
C20:4 (arachidonic acid)		
Mean (SD)	8.7 (2.1)	108.1 (31.3)
Median (Q1-Q3)	8.4 (7.6-9.7)	106.6 (85.9-124.6)
Min-Max	4.3-17.0	45.0-245.8
Missing	0	0
C20:5 (eicosapentenoic acid)		
Mean (SD)	0.6 (0.5)	7.2 (5.s4)
Median (Q1-Q3)	0.5 (0.4-0.7)	5.9 (4.3-8.0)
Min-Max	0.2-4.5	2.0-41.3
Missing	0	0
FFA	Relative concentration (%)	Absolute concentration (ng/mL)
C16:0 (palmitic acid)		
Mean (SD)	33.8 (3.5)	147.1 (33.3)
Median (Q1-Q3)	33.8 (33.1-35.0)	144.5 (124.6-164.2)
Min-Max	2.1-44.0	6.2-263.5
Missing	0	0
C16:1 (palmitoleic acid)		
Mean (SD)	1.4 (0.4)	6.2 (3.1)
Median (Q1-Q3)	1.3 (1.1-1.7)	5.7 (4.2-7.8)
Min-Max	0.6-2.7	2.1-21.8
Missing	0	0
C18:2 (linoleic acid)		
Mean (SD)	18.9 (2.5)	81.9 (17.1)
Median (Q1-Q3)	19.1 (18.1-20.2)	82.0 (72.1-91.5)
Min-Max	0.4-24.4	1.0-130.5
Missing	0	0
C20:4 (arachidonic acid)		
Mean (SD)	5.2 (1.0)	22.4 (6.0)
Median (Q1-Q3)	5.0 (4.6-5.7)	22.0 (18.9-23.9)
Min-Max	1.5-8.8	5.1-55.1
Missing	0	0
C20:5 (eicosapentenoic acid)		

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Table 3 (*continued*)

Variable	N = 112 (%)	
EFA	**Relative concentration(%)**	**Absolute concentration (µg/mL)**
Mean (SD)	0.3 (0.2)	1.1 (0.7)
Median (Q1-Q3)	0.2 (0.1-0.3)	0.8 (0.6-1.2)
Min-Max	0.0-1.0	0.1-5.0
Missing	0	0
C22:6 (docosahexaenoic acid)		
Mean (SD)	1.0 (0.5)	4.6 (2.3)
Median (Q1-Q3)	1.0 (0.7-1.3)	4.1 (3.0-6.0)
Min-Max	0.0-1.0	0.5-13.2
Missing	0	0

Abbreviations: EFA = esterified fatty acids; FFA = free fatty acids; N = number; Q1-Q3 = inter-quartile range; SD = standard deviation.

Table 4 Multivariable Analysis for PFS and OS

Variable (N = 112)	HR (95% CI)	*P* value of contrasts	*P* value	HR (95% CI)	*P* value of contrasts	*P* value
	PFS			**OS**		
Line of IO	-	-	<0.001	-	-	<0.001
1	0.20 (0.10-0.39)	<0.001	-	0.18 (0.08-0.40)	<0.001	-
2	0.76 (0.42-1.37)	0.360	-	1.36 (0.73-2.51)	0.332	-
>2	reference	-	-	reference	-	-
ECOG PS	-	-	0.010	-	-	<0.001
0	0.53 (0.32-0.86)	0.010	-	0.35 (0.20-0.63)	<0.001	-
1	reference	-	-			
Histology	-	-	0.954	-	-	0.047
Non-squamous	0.98 (0.57-1.69)	0.954	-	0.66 (0.37-1.16)		-
Squamous	reference	-	-	reference	-	-
Smoking status	-	-	0.092			
Current/former smoker	0.72 (0.92-3.24)	0.092	-	*Not selected*		
Never smoker	reference	-	-			
Age		*Not selected*		0.97 (0.95-1.00)	0.047	0.047
NLR	1.15 (1.09-1.21)	<0.001	<0.001	1.19 (1.12-1.27)	<0.001	<0.001
EFA C16:0 (%)	1.16 (1.04-1.30)	0.008	0.008	*Not selected*		
EFA C16:1 (%)	0.68 (0.48-0.96)	0.030	0.030	*Not selected*		
EFA C16:1 (µg/mL)	*Not selected*			0.97 (0.95-0.99)	0.001	0.001
FFA C16:0 (%)	0.91 (0.95-0.97)	0.005	0.005	*Not selected*		
FFA C18:0 (%)	*Not selected*			0.73 (0.55-0.95)	0.020	0.020

Abbreviations: CI = confidence interval; ECOG PS = Eastern Cooperative Oncology Group performance status; EFA = esterified fatty acid; FFA = free fatty acid; HR = hazard ratio; IO = immunotherapy; NLR = neutrophil/lymphocyte ratio; Not selected = variable considered in the stepwise process, but not selected for the inclusion in the final multivariable model; OS = overall survival; PFS = progression-free survival.

global quantity of a FA in the organism; it is more variable than relative concentration, both in time, and among individuals.[32] Furthermore, total FA concentration has been measured as the sum of 2 circulating components: the esterified and free one. FFAs represent an essential source of lipid fuel; they are also agonists of G protein-coupled receptors involved in metabolic and immune modulation.[33] FFAs are principally mobilized by peripheral adipose tissue in conditions of an energetic requirement (eg, intense physical effort, prolonged starvation). However, also endothelial lipases contribute to the FFA pool from chylomicrons, especially under a high-fat diet, and in minor contribution from VLDL.[34] On the contrary, EFAs represent the circulating fraction esterified in triglycerides, phospholipids, cholesterol esters, and sphingolipids associated with lipoproteins. EFAs and FFAs are subject to different metabolic regulations: EFAs are linked to comprehensive individual metabolic profile and liver activity; FFA ranges are highly variable, as they reflect the individual energetic needs.

At this regard, the carrier role of triglycerides for circulating FAs is in line with the strong positive correlation we observed between such variables, including C16:0, C16:1, and C18:0. Furthermore, both EFAs and FFAs correlated with total cholesterol, confirming that these components of the circulating FA pool are associated to lipoprotein level and composition. The absence of strong correlations between FAs and main clinical variables (age, BMI,

histology, number of metastatic sites, and smoking status) suggests that their levels are relatively independent of these factors. On the contrary, some relevant associations emerged between several FAs and gender. This intriguing observation seems to confirm the existing evidence that gender differences impact FA metabolism,[35] sustaining that a personalized management should also consider this variable. However, an in-depth discussion on the significance of these correlations goes beyond the purpose of this manuscript.

When performing multivariable analyses on cholesterol, triglyceride, and FA circulating levels, the stepwise selection process evidenced a prognostic role of FFA and EFA C16:0, and EFA C16:1 in terms of PFS; a prognostic role of EFA C16:1 and C18:0 in terms of OS. C16:0 (palmitate) is a saturated FA with a wide distribution in food (eg, vegetal oil, meat, butter, and cheese). While its role in increasing the risk of cardiovascular disease is clear, data about its association with cancer are inconclusive.[36] C16:1 (palmitoleic acid) and C18:0 (stearic acid) can be assumed with food (eg, marine and vegetal oils, milk) but are mostly synthesized by the liver starting from palmitate. Data on cancer also lack for these FAs. C16:1 and C18:0 are associated with a more favorable metabolic profile than palmitic acid, with a lower LDL increase and a protective effect on endothelial inflammation.[37,38]

The prognostic effect of some FAs upon IO could be explained by 1 or more of the many mechanisms through which lipid metabolism modulates immunity (eg, modification of mitochondrial activity, synthesis of inflammatory mediators, influence on receptor expression, and clustering). Among them, a particularly intriguing hypothesis involves the effect of FAs on cell membranes. All FA plasmatic concentration modifications induce parallel membrane composition changes, measurable in different cell types, including red blood cells.[39] In immune cells, imbalances in membrane fluidity can entail a pleiotropic effect on proliferation, differentiation, and activation pathways. IO itself exerts its impact on anti-cancer immunity through interference with membrane processes (eg, PD-1/PD-L1 axis). On this basis, we may hypothesize that the observed prognostic role of EFA C16:0, C16:1, and C18:0 is likely at least partially mediated by their structural effect within immune cell membranes. In this case, the increase in EFA C16:0 content can reflect an augmented membrane saturation level, with stiffening of lipid rafts. On the contrary, high levels of EFA C16:1 and C18:0 can increase membrane fluidity by adding a double binding between 2 carbons (C16:1) and elongating the 16-carbon chain (C18:0). Furthermore, C16:1 and C18:0 are synthesized mainly by the liver starting from C16:0. Therefore, their increase is directly associated with a decrease in their precursor. In all these 3 cases, the structural role in membrane composition is a prerogative of EFAs. This could explain the apparent opposite prognostic role of EFA and FFA C16:0, as the FFA component is mobilized to sustain fatty acid oxidation; on the other hand, the EFA 1 is involved in the constitution of the membranes.[40]

A recent work by Yao et al. supports this mechanism for C16:0 and PD1/PD-L1 axis. In brief, the authors report that PD-L1 on the cancer cell surface can be palmitoylated in its cytoplasmic domain. This modification stabilizes the receptor, blocking its ubiquitination, and inhibiting lysosomal degradation. The stabilization of membrane PD-L1 counteracts the inhibition by anti-PDL1 agents, as the receptor blockage is balanced by intra-cellular storage and active redistribution. ZDHHC3 (DHHC3) is the specific acyltransferase responsible for palmitoylation. Its selective inhibition restores tumor sensitivity to IO in mice models.[20] If confirmed in humans, this could be a plausible mechanism to explain the unfavorable prognostic effect of EFA C16:0 observed in our case series. Notably, all our patients were treated with IO agents acting on different steps of the PD-1/PD-L1 axis, potentially subject to such kind of modulation.

Our work presents several limitations. It is a single Institution case series with a relatively limited number of patients. Its retrospective nature impeded the evaluation of some potentially interesting clinical variables, such as the influence of diet on individual lipid profile. Results could not receive external validation on an independent case series. IO treatment is not homogeneous, considering both different lines and agents. All patients received IO alone, so that no data can be derived on lipid metabolism during combined chemo-IO or other kinds of therapies for NSCLC. Furthermore, only EFA C16:1 was associated with PFS and OS, while FFA C16:0 was related to PFS and EFA C18:0 to OS. Nonetheless, this work provides an insight into a poorly explored field of cancer IO. To our knowledge, no studies have been conducted to investigate the impact of lipid metabolites on IO efficacy in NSCLC. The topic of immune modulation by lipid mediators represents a very innovative field with potential therapeutic implications.

Conclusions

The results of this study support the role of some lipid variables in modulating the efficacy of IO in advanced NSCLC. Analyzing the biological activity of relevant FAs, data focused on modifications in membrane composition. If confirmed, these data may support the development of metabolic reprogramming strategies through either a dietetic or pharmacologic intervention to boost IO activity. Given the potential broad applicability of this approach, the topic of lipid metabolism in anti-cancer immunity de-serves further investigation.

Clinical practice points

- Preclinical data suggest that lipid metabolism, particularly fatty acid balance, modulates the individual sensitivity to cancer immunotherapy. However, evidence lacks in humans. We aimed to investigate the prognostic impact of circulating FAs in advanced non-small cell lung cancer (NSCLC) patients treated with immunotherapy. With this purpose, we dosed a broad panel of free and esterified fatty acids at IO baseline in a retrospective cohort of advanced NSCLC patients. We evidenced that some esterified unsaturated (C16:1) and middle-chain (C18:0) fatty acids have a positive prognostic role. On the contrary, their esterified saturated counterpart (C16:0) has a negative prognostic impact. This is the first evidence in humans supporting the hypothesis that lipid metabolism may influence the outcome upon IO of NSCLC patients. If confirmed, our data may open innovative perspectives to boost the efficacy of IO through a metabolic intervention.

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Disclosure

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: C.P., travel accommodations and honoraria with MSD International GmbH, BMS, Eli Lilly; G.L.R., travel accommodations and honoraria with AstraZeneca, MSD International GmbH, BMS, Eli Lilly; D.S., travel accommodations and honoraria with AstraZeneca, MSD International GmbH, BMS; F.D.B., consultation, advisory boards and/or lectures, honoraria or educational grants with Amgen, AstraZeneca, Boehringer-Ingelheim, BMS, Eli Lilly, F. Hoffmann-La Roche, Ignyta, Merck Sharp and Dohme, Merck Serono, Novartis, Pfizer; M.C.G., personal financial interests with AstraZeneca, MSD International GmbH, BMS, Boehringer Ingelheim Italia S.p.A, Celgene, Eli Lilly, Ignyta, Incyte, Inivata, MedImmune, Novartis, Pfizer, Roche, Takeda, Institutional financial interests with Eli Lilly, MSD, Pfizer (MISP), Astra-Zeneca, MSD International GmbH, BMS, Boehringer Ingelheim Italia S.p.A, Celgene, Ignyta, Incyte, Inivata, MedImmune, Novartis, Pfizer, Roche, Takeda, Tiziana, Foundation Medicine, research funding from AIRC, AIFA, Italian Ministry of Health, TRANSCAN; all the other authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Supplementary materials

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