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The influence of nutritional status, lipid profile, leptin concentration and polymorphism of genes encoding leptin and neuropeptide Y on the effectiveness of immunotherapy in advanced NSCLC patients

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Abstract

Introduction Neuropeptide Y is a neurotransmitter in the nervous system and belongs to the orexigenic system that increases appetite. Its excessive secretion leads to obesity. Leptin is a pro-inflammatory adipokine (produced in adipose tissue) induced in obesity and may mediate increased antitumor immunity in obesity (including the promotion of M1 macrophages). *Leptin* and *neuropeptide Y* gene polymorphisms, causing increased leptin levels and the occurrence of obesity, and lipid profile disorders, may increase the effectiveness of immunotherapy.

Materials and methods In 121 patients with advanced NSCLC without mutations in the *EGFR* gene and rearrangements of the *ALK* and *ROS1* genes, undergoing immunotherapy (1st and 2nd line of treatment) or chemoimmunotherapy (1st line of treatment), we assessed BMI, lipid profile, PD-L1 expression on cancer cells using the immunohistochemical method (clone SP263 antibody), leptin concentration in blood serum by ELISA, polymorphisms in the promoter region of the genes for *leptin* (*LEP*) and neuropeptide Y (*NPY*) by real-time PCR.

Results Leptin concentration was significantly higher in obese patients than in patients with normal or low weight ($p=0.00003$) and in patients with disease stabilization compared to patients with progression observed during immunotherapy ($p=0.012$). Disease control occurred significantly more often in patients with the GA or AA genotype than patients with the GG genotype in the rs779039 polymorphism of the *LEP* gene. The median PFS in the entire study group was five months (95% CI: 3-5.5), and the median OS was 12 months (95% CI: 8–16). Median PFS was highest in patients with TPS ≥ 50% (6.5 months) and in obese patients (6.6 months). Obese patients also had a slightly longer median OS compared to other patients (23.8 vs. 13 months). The multivariate Cox logistic regression test showed that the only factor reducing the risk of progression was TPS ≥ 50% (HR = 0.6068, 95% CI: 0.4001–0.9204, $p=0$,

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0187), and the only factor reducing the risk of death was high leptin concentration (HR = 0.6743, 95% CI: 0.4243–1.0715, $p = 0.0953$).

Conclusion Assessment of nutritional status, serum leptin concentration and polymorphisms in the *LEP* gene may be of additional importance in predicting the effectiveness of immunotherapy and chemoimmunotherapy in patients with advanced NSCLC.

Keywords Non-small cell lung cancer, Immunotherapy, Obesity, Lipid profile, Leptin

Introduction

Immune checkpoint inhibitors (ICIs) such as monoclonal antibodies against programmed death receptor 1 (PD-1, CD279), programmed death ligand 1 (PD-L1, CD274) cytotoxic T cell antigen 4 (CTLA-4) or lymphocyte-activation gene 3 (LAG-3) widely used in the systemic treatment of different cancers. Cancer cells can develop an increased expression of negative immune checkpoints on their surface and inactivate cytotoxic T-cells [1]. As a result, T-cells do not recognise or destroy cancer cells, and the entire tumor microenvironment (TME) is poor in immune cells and pro-inflammatory cytokines [2]. It is one of the tumor's mechanisms of escaping from immune surveillance, ensuring the prolongation of its existence and progression. Immunotherapy involves using monoclonal antibodies against molecules on the surface of immune system cells or cancer cells, causing the reactivation of T lymphocytes and initiating the process of tumor destruction [3, 4]. It is believed that immunotherapy is currently the only form of systemic treatment that can potentially lead to complete cancer regression, regardless of the stage of advancement.

Lung cancer has been one of the most common malignant tumors in the world and the leading cause of death for years. In 2022, Globocan registered 2.48 million new cases of lung cancer worldwide, and 1.82 million patients died [5]. The most common lung cancer is non-small cell cancer (NSCLC) - it accounts for 85% of all lung cancer patients. Numerous studies have confirmed the greater effectiveness of immunotherapy compared to chemotherapy in treated NSCLC patients, both in first and second line systemic therapy, as well as in perioperative treatment [6–9]. The most investigated predictive factor of the effectiveness of immunotherapy in lung cancer is the expression of PD-L1 on tumor cells described also as tumor proportion score (TPS). Cancer cells produce on their surface a PD-L1 and inhibit immune cells with PD-1 expression. PD-1 is located on the surface of T and B lymphocytes, macrophages and monocytes, and its activation causes suppression of the immune system [3]. The use of monoclonal antibodies breaks the mechanism of tumor escape from the host's immune surveillance. A high TPS is associated with higher effectiveness of immunotherapy using anti-PD-1 monoclonal antibodies - pembrolizumab, nivolumab and cemplimab, and anti-PD-L1 antibodies -- atezolizumab, durvalumab and avelumab used in monotherapy or combination. However, not every patient with high TPS showed satisfactory effectiveness of immunotherapy, indicating the presence of other factors important in the tumor escape from immune surveillance. In searching for other predictive factors, attention was drawn to the correlation between the effectiveness of immunotherapy and body weight, lipid metabolism, serum leptin and neuropeptide Y concentration as well as gene polymorphisms.

Cancer cachexia is a well-recognized poor prognostic factor in different neoplasms. Poor performance status and chronic inflammation (e.g. production of large amounts of TNF-α by the immune cells) are associated with cancer cachexia. Normal or excessive body weight is associated with longer survival of cancer patients. It is unclear whether the increased effectiveness of immunotherapy in patients with advanced NSCLC with normal body weight or obesity is related to their better performance status or the immunomodulatory effect of peptides regulating nutritional status. It has been proven that high leptin concentrations observed in obesity are associated with the activation of the immune system, including the production of interferons, which may increase the effectiveness of immunotherapy [10]. Moreover, the LDL cholesterol fraction increases significantly in obese patients. Cholesterol induces immune cell differentiation and function and plays important roles in monocyte priming, neutrophil activation, hematopoietic stem cell mobilization, and enhanced T cell production, which may also affect the effectiveness of immunotherapy [11].

Obese patients showed an increased number of mutations in genes encoding the lipid metabolism pathway, a high number of macrophages as well as CD4+and CD8+T cells, a high concentration of interferon-γ and enhanced immunogenic factors such as tumor mutational burden (TMB). A correlation has been demonstrated between a high number of mutations and prolonged progression-free survival in NSCLC patients receiving immune checkpoint inhibitors, indicating that this marker can be used as a predictive factor for these patients [12]. The above phenomenon is the "obesity paradox", which consists in the surprisingly good effectiveness of immunotherapy in obese patients, and it is the

subject of extensive research around the world among patients with various types of cancer [13].

Leptin is a polypeptide hormone product of the *obese* (*Ob*) or *leptin* (*Lep*) gene with a specific weight of 16 kDa, consisting of 167 amino acids. It is synthesized mainly in white (subcutaneous) adipose tissue [14]. Leptin is additionally synthesized in the intestines, where it is released into the intestinal lumen and can enter the systemic circulation. It is also produced by skeletal muscle in response to exogenous leptin and as a result of hyperglycemia and hyperlipidemia. Plasma leptin concentration is usually proportional to body fat mass, is higher in women, and has a circadian rhythm (higher at night) [15, 16]. Leptin is crucial in regulating metabolism, assuring homeostasis and body weight control through a negative biofeedback mechanism between adipose tissue and the hypothalamus.

Leptin receptor (known as Ob-R or LEP-R) is expressed in immune cells. Therefore leptin is considered a link between metabolism and the immune system and is involved in inflammatory processes [17].

Leptin inhibits food intake and reduces appetite and weight loss (anorexigenic system), but it also inhibits the synthesis and secretion of neuropeptide Y, which has a strong stimulating effect on food intake (orexigenic system) [18]. By inhibiting the release of neuropeptide Y (NPY), leptin increases thermogenesis and energy expenditure, activates lipolysis and inhibits lipogenesis. In obesity, the rhythm of leptin release is disturbed, and its level is generally and chronically raised (hyperleptinemia) [14]. Therefore, mutations and polymorphisms in the leptin gene, associated with lacking or disturbing leptin activity, may lead to obesity.

Leptin exhibits broad immunomodulatory properties [19, 20]. Leptin increases the production of GM-CSF (granulocyte-macrophage colony-stimulating factor) and G-CSF in the bone marrow, activates macrophages and monocytes, and intensifies the phagocytosis [14, 21, 22]. It also participates in the induction of proliferation, differentiation, and activation of T and B lymphocytes, as well as the development and cytotoxicity of NK cells. It induces the expression of pro-inflammatory cytokines such as IL-6, IL-8, IL-12, IL-18, and TNF-α in tumor microenvironment (TME). It timulates the production of reactive oxygen species and chemotaxis in multinucleated cells (neutrophils, basophils, eosinophils) [23–27]. These molecules contribute to the creation of chronic, low-intensity inflammation. Neuropeptide Y, a 36-amino acid peptide, is one of the most widely distributed neuropeptides in the brain. It plays an important role in orexigenic (appetite-stimulating) processes and regulates the hormone secretion from the anterior pituitary gland. Inhibition of the NPY reduces obesity and other endocrine alterations resulting from chronic leptin deficiency. On the other hand, leptin decreases the expression of the *NPY* gene in NPY neurons [15].

Our study analyzed the correlation between body weight, lipid profile, serum leptin concentration and immunotherapy's effectiveness in patients with advanced non-small cell lung cancer. We also analyzed the effect of polymorphisms of genes encoding leptin and NPY on treatment effectiveness.

Materials and methods

Characteristics of the studied group

Two-centre, prospective, non-randomized study enrolled 121 patients (median age 68±6.7 years, 71 males and 50 females) with locally advanced (stage IIIB −9 patients) or advanced (stage IV −112 patients) NSCLC. The presence of mutations in the *EGFR* (*epidermal growth factor receptor*) gene and rearrangement of the *ALK* (*anaplastic lymphoma kinase*) and *ROS1* (*ROS1 protooncogene*) genes were excluded before qualification for the treatment. Fifty-six patients (46.3% of patients) showed TPS≥50%, of which 51 patients received pembrolizumab in the first line of treatment. Twenty-one patients with TPS<50% received chemotherapy combined with pembrolizumab. 49 patients received second line immunotherapy, regardless of the status of PD-L1 expression (in 5 patients were: TPS≥50%;, in 4 patients, it was not tested; and in 40 patients TPS<50%). To assess body weight, the body mass index (BMI) was used: BMI<18.5 is classified as underweight, BMI≥18.5 and <25 is classified as normal weight, BMI≥25 and <30 is classified as overweight (obesity class I: BMI≥30 and <35, obesity class II: BMI≥35 and <40, obesity class III: BMI≥40). Obesity at the time of qualification for treatment was observed in 26 patients (21.5% of patients), and overweight in 39 patients (32.2%). Lipid profile (total cholesterol, triglycerides, cholesterol fractions: LDL, HDL, non-HDL) was examined in all patients as part of routine laboratory tests before the start of treatment. Serum leptin concentration was determined by ELISA (enzyme-linked immunosorbent assay) in 119 patients. *LEP* (rs779039 and rs 21672770) and *NPY* (16138, rs 16478) gene polymorphisms were tested by real-time PCR (polymerase chain reaction) in DNA isolated from peripheral blood leucocytes in 116 patients. Response to immunotherapy, progression-free survival and overall survival calculated from the start of therapy were assessed in all patients. The percentage of patients with 6-month progression-free survival and with 6-month overall survival was also determined. One hundred two patients (84.3%) discontinued immunotherapy due to progression or toxicity of treatment, and 80 deaths (66.1% of patients) were reported at the end of follow-up. The characteristics of the study group are presented in Table 1.

Table 1 Characteristic of population. Abbreviation: NSCLC NOS – non-small cell lung cancer not otherwise specified, PD-L1 – programmed death ligand 1, TC – tumor cells, BMI – body mass index, PR – partial response, SD – stable disease, PD- progression disease, PFS – progression free survival, OS – overall survival, *LEP* – leptin gene, *NPY* – neuropeptide Y gene

Characteristic		Value
Gender (n, %)	Female	50 (41.3%)
	Male	71 (58.7%)
Age (median ± SD)		68 ± 6.7 years
Pathomorphological diagnosis (n, %)	Adenocarcinoma	62 (51.2%)
	Squamous cell carcinoma	50 (41.3%)
	Large cell carcinoma	2 (1.7%)
	NSCLC NOS	7 (5.8%)
Stage of disease (n, %)	IIIB	9 (7.4%)
	IV	112 (92.3%)
Percentage of TC with PD-L1 expression (median ± SD)		30 ± 32%
PD-L1 expression (n, %)	≥ 1% of TC	97 (80.2%)
	< 1% of TC	20 (16.5%)
	≥ 50% of TC	56 (46.3%)
	< 50% of TC	61 (50.4%)
	Data not available	4 (3.3%)
Type of treatment (n, %)	First line pembrolizumab	51 (42.2%)
	First line pembrolizumab plus chemotherapy	21 (17.4%)
	Second line nivolumab	22 (18.2%)
	Second line atezolizumab	27 (22.3%)
BMI (median ± SD)		25.15 ± 4.55
Nutritional status (n, %)	Below normal weight (BMI < 18,5)	2 (1,65%)
	Normal (BMI ≥ 18,5 i < 25)	56 (46.3%)
	Overweight (BMI ≥ 25 i < 30)	39 (32.2%)
	Obesity (BMI ≥ 30)	26 (21.5%)
	Obesity I class (BMI ≥ 30 and < 35)	23 (19,0%)
	Obesity II class (BMI ≥ 35 and < 40)	2 (1,65%)
	Obesity III class (BMI ≥ 40)	1 (0,82%)
Total cholesterol (median ± SD)		168 ± 48.13 mg/dL
LDL (median ± SD)		103 ± 40.12 mg/dL
Non-HDL (median ± SD)		122 ± 41.8 mg/dL
HDL (median ± SD)		43 ± 13.53 mg/dL
Leptin (median ± SD)		13.86 ± 22.44 µg/L
rs779039 of *LEP* gene (n, %)	GG	22 (18.2%)
	GA	87 (71.9%)
	AA	7 (5.8%)
	Data not available	5 (4.1%)
rs2167270 of *LEP* gene (n, %)	GG	46 (38.0%)
	GA	53 (43.8%)
	AA	17 (14.1%)
	Data not available	5 (4.1%)
rs16138 of *NPY* gene (n, %)	GG	60 (49.6%)
	GC	56 (46.3%)
	Data not available	5 (4.1%)
rs16478 of *NPY* gene (n, %)	GG	55 (45.5%)
	GA	61 (50.4%)
	Data not available	5 (4.1%)
Response to treatment (n, %)	PR	34 (28.1%)
	SD	36 (29.8%)
	PD	51 (42.1%)
Median PFS (95% CI)		5 (3.0-5.5) months

Table 1 (continued)

Characteristic		Value
Six-months PFS (n, %)	< 6 months	74 (61.2%)
	≥ 6 months	47 (38.8%)
Patients on treatment at the end of the observation (n, %)	Yes	19 (15.7%)
	No	102 (84.3%)
Median OS (95% CI)		12 (8.0–16.00) months
Six months OS (n, %)	< 6 months	40 (33.1%)
	≥ 6 months	81 (66.9%)
Deaths at the end of the observation (n, %)	Yes	80 (66.1%)
	No	41 (33.9%)

All patients gave their written consent to participate in the study. The study was approved by the local Bioethics Committee at the Medical University of Lublin (approval number – KE-0254/95/2018).

Nutritional status and lipid profile evaluation

Sample collection

The blood samples were collected into the EDTA tubes and centrifuged in 2000 x g for 10 min. Immediately, plasma was collected and stored at -80ºC until the ELISA test was performed. The remaining blood was stored at -80ºC until DNA was isolated.

Enzyme-linked immunosorbent assay (ELISA) for assessment of leptin concentration

To detect leptin concentration in plasma samples an ELISA test (Life Technologies Corporation, Invitrogen, USA; Catalog Number; KAC2281) was applied. The ELISA assay was performed according to the manufacturer's instructions. The plate was read at 450 nm on BioTek ELx800 Absorbance Microplate Reader (BioTek, Winooski, VT, USA). A standard curve was generated, from which concentration results were obtained. Analysis was performed with Gen5 3.03 Microplate Reader and Imager Software (BioTek, Winooski, VT, USA).

Real-time PCR method for LEP and NPY gene polymorphism examination

DNA was isolated using a Qiamp DNA blood kit (Qiagen, Germany) according to the manufacturer's instructions. Isolated DNA was stored at -80ºC until quantitative PCR (qPCR) was performed. qPCR was applied to detect polymorphisms of *LEP* gene (rs7799039 and rs2167270) and *NPY* gene (rs16138 and rs16478).

PCR mixture contained 7.5 µl of genotyping master mix (Applied Biosystems; ThermoFisher Scientific, Inc., USA), 2 µl of genomic DNA isolated from the whole blood (10 ng/µl) and 0.5 µl of TaqMan SNP assay (Applied Biosystems; ThermoFisher Scientific, Inc. USA). The following conditions were applied to perform the qPCR reaction: 95 °C for 10 min, and the subsequent 40 cycles of 95 °C for 15 s and 62 °C for 60 s. qPCR was performed on Illumina Eco Real-Time PCR equipment (Illumina, Inc., USA).

Statistical analysis

Data were entered as numbers and percentages (for categorized variables), as well as medians and standard deviations (SD) (for continuous variables). Pearson's chi-square test was used to compare the characteristics of the groups divided according to response to treatment as well as the presence of six-month PFS and OS. We used the U-Mann Whitney test for testing the equality of population medians among groups with different demographic and clinical factors, lipid profile, serum concentration of leptin as well as polymorphisms of *LEP* and *NPY* genes. Differences in BMI medians before and during treatment were estimated using the Wilcoxon test. Spearman's correlation was used to measure the strength and direction of association between two ranked variables. Kaplan-Meier analysis was used to compare these groups' progression-free survival and overall survival. The Cox regression model with a stepwise selection procedure was used to establish a predictive model for NSCLC patients treated with immunotherapy. These tests were performed with Statistica v. 13.1 (Tibco Software, USA) and MedCalc (MedCalc Software Ltd, Ostend, Belgium) software. The p-value was considered significant if it was less than 0.05.

Results

Lipid profile association with demographic and clinical characteristics as well as *LEP* and *NPY* genes polymorphisms

Significantly higher concentration of HDL ($p=0.0327$) and leptin ($p=0.0004$) was found in female compared to male patients. Concentrations of total cholesterol ($p=0.0042$), LDL ($p=0.006$), non-HDL and triglycerides ($p=0.0069$) were significantly higher in younger patients (<65 years of age) than in older patients (≥65 years of age). HDL was higher in non-SCC compared to SCC patients ($p=0.0013$). Obese and overweight patients had significantly higher leptin concentrations compared

to normal-weight patients ($p=0.00003$, $p=0.0072$, respectively).

In the group of patients with TPS≥1%, significantly lower HDL concentration ($p=0.0042$) and significantly higher triglyceride concentration ($p=0.05$) were found compared to the group of patients with TPS<1%. In contrast, cholesterol ($p=0.0019$), LDL (0.012) and non-HDL ($p=0.006$) levels were significantly lower in patients with TPS≥50% than in patients with TPS<50% (Fig. 1a).

Leptin concentration was significantly higher ($p=0.012$) in patients with stable disease compared to patients with disease progression during immunotherapy. The percentage of tumor cells with PD-L1 expression was significantly higher ($p=0.0182$) in patients with PFS longer than six months than in patients with shorter PFS.

Patients with the GG genotype of the rs779039 polymorphism of the *LEP* gene had a significantly higher percentage of tumor cells with PD-L1 expression compared to patients with the genotypes GA ($p=0.0052$), AA ($p=0.0242$) and GA+AA ($p=0.0038$) (Fig. 1b). The remaining polymorphisms did not affect the evaluated features.

Percentage of tumor cells with PD-L1 expression correlated significantly negatively with concentrations of total cholesterol (R=-0.2638, $p=0.0044$), LDL (R=-0.2622, $p=0.0056$), non-HDL (R=-0.2112, $p=0.0247$), HDL (R=-01918, $p=0.041$) and significantly positively with triglyceride concentration (R=+0.1838, $p=0.0492$). Leptin concentration significantly positively correlated with BMI (R=+0.4958, $p<0.000001$) and with HDL concentration ($R=0.1873$, $p=0.0441$). Positive correlations were also observed between total cholesterol concentration,, its fractions and triglycerides.

The relationship between different clinical and demographic characteristics, lipid profile, genetic polymorphisms and the effectiveness of immunotherapy

Response to treatment occurred in 34 patients (28.1%), stable disease - in 36 patients (29.8%) and disease progression - in 51 patients (42.1%). The median PFS was five months (95% CI: 3-5.5), and the median overall survival (OS) was 12 months (95% CI: 8–16). The group of patients with an inferior prognosis were those who received chemoimmunotherapy. Seven patients (33.3%) in this group did not receive a second cycle of chemotherapy due to toxicity grade 3–4. Lack of toxicity of chemotherapy and high expression of PD-L1 on TC was probably associated with a higher median PFS in patients treated with pembrolizumab (6.5 months, 95% CI: 4.0–12.0) compared to patients receiving pembrolizumab in combination with chemotherapy (2.8 months, 95% CI: 2-6.5). The median of PFS was 5.0 months in patients treated with nivolumab (95% CI: 2.6–8.5) and 3.5 months in patients who received atezolizumab (95% CI: 2.5–6.5). Similarly, median OS was higher in patients treated with pembrolizumab (23.8 months, 95% CI: 7.5–30.5) than in patients who received chemoimmunotherapy (7.5 months, 95% CI: 1.8–12.5), nivolumab (13.5 months, 95% CI: 8.5–17) and atezolizumab (16.5 months, 95% CI: 7-23.5).

Disease control was significantly more frequent ($\chi^2=4.131$, $p=0.0421$) in patients with the GA+AA genotype (65.5%) compared to carriers of the GG genotype (38.1%) in the rs779039 polymorphism of the *LEP* gene. In addition, the percentage of patients with PFS longer than six months was slightly higher ($\chi^2=2.88$, $p=0.0897$) in the group of patients with TPS≥50% (46.4%) than in the group of patients with TPS<50% (31.15%). The percentage of patients with overall survival over six months was similar in all analyzed groups. Lipid profile, leptin concentration, and obesity did not afectthe rate of

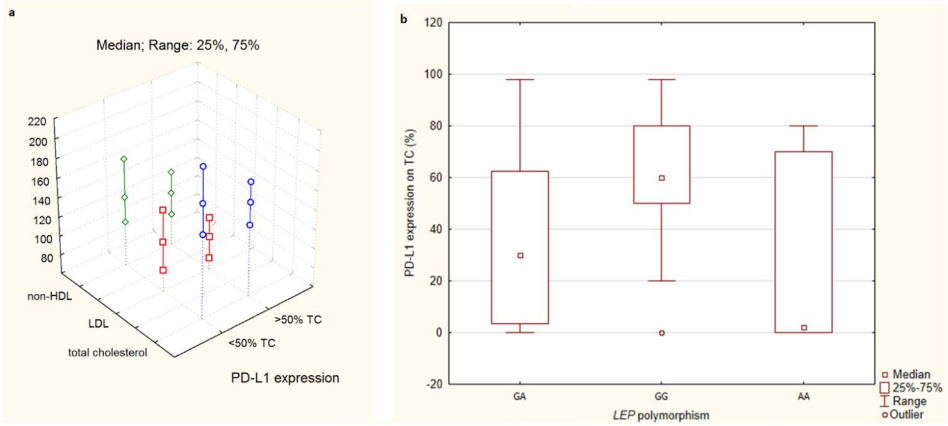

Fig. 1 (**A**) Concentration (mg/dL) of total cholesterol, LDL and non-HDL in patients with TPS < 50% (0) and in patients with TPS ≥ 50% (1). (**B**) TPS in patients with the different genotypes of rs779039 polymorphism in the LEP gene

patients who were disease-controlled, progression-free or alive for more than six months (Table 2).

BMI decreased insignificantly after the first three months of immunotherapy. The median BMI before immunotherapy was 25.15±4.55, and during the first control of treatment −24.67±4.5 (p=0.1834). Progression free survival and overall survival were similar in all analyzed groups. Median PFS was only numerically higher in patients with TPS 50% (6.5 months, 95% CI: 4–12) than in patients with lower PD-L1 expression (4 months, 95% CI: 2.55–5.5) and obese patients (6.6 months, 95% CI: 3–12) than in patients with lower body weight (4.3 months, 95% CI: 3-6.5). Obese patients also showed insignificantly higher median OS (23.8 months,

Table 2 Response to immunotherapy, six-month PFS and six-month OS according to demographic factors, clinical factors and lipid profile. Abbreviation: SCC – squamous cell carcinoma, TC – tumor cells, UPR – upper reference limit, *LEP* – leptin gene, *NPY* – neuropeptide Y gene

Population characteristic		Progression disease (*n*, %)	Disease control (*n*, %)	χ^2, *p*	PFS < 6 months	PFS ≥ 6 months	χ^2, *p*	OS < 6 months	OS ≥ 6 months	χ^2, *p*
Whole group		51 (42.1)	70 (57.9)	-	74 (61.2)	47 (38.8)	-	40 (33.1)	81 (66.9)	-
Gender	Female	19 (38)	31 (62)	0.602	29	21	0.358	15 (30)	35 (70)	0.36
	Male	32 (45.1)	39 (54.9)	0.4378	45	26	0.5496	25 (35.2)	46 (64.8)	0.5485
Age	< 65 years	22	20	2.762	25	16	0.001	11	30	1.087
	≥ 65 years	29	50	0.0965	49	31	0.9748	29	51	0.2971
Diagnosis	Non-SCC	33	38	1.321	44	27	0.048	25	46	0.36
	SCC	18	32	0.2504	30	20	0.8266	15	35	0.5485
Stage of disase	IIIB	4	5	0.021	7	2	1.131	4	5	0.57
	IV	47	65	0.8848	67	45	0.2876	36	76	0.4503
PD-L1 expression	< 1% of TC	11	9	1.706	14	6	0.73	4 (20)	16 (80)	2.158
	≥ 1% of TC	38	59	0.1915	58	39	0.3929	36 (37.1)	61 (62.9)	0.1418
PD-L1 expression	< 50% of TC	28	32	1.159	42	19	**2.88**	21	40	0.003
	≥ 50% of TC	21	36	0.2817	30	26	**0.0897**	19	37	0.9563
Line of treatment	First line	25	24	2.175	42	28	0.094	26	44	1.252
	Second line	27	45	0.1406	32	19	0.7591	14	37	0.2632
Obesity	No	41	54	0.614	61	34	1.735	31	64	0.036
	Yes	9	17	0.4333	13	13	0.1878	9	17	0.8495
Cholesterol	Normal	30	17	0.487	47	30	0.001	25	52	0.033
	URL	20	24	0.4853	27	17	0.9748	15	29	0.8558
Triglyceride	Normal	32	52	1.851	54	30	1.132	27	57	0.104
	URL	19	18	0.1737	20	17	0.2873	13	24	0.7471
LDL	Normal	39	54	0.007	57	36	0.003	31	62	0.007
	URL	12	16	0.9333	17	11	0.9563	9	19	0.9333
Non-HDL	Normal	32	38	0.508	46	24	1.452	23	47	0.003
	URL	20	31	0.476	28	23	0.2282	17	34	0.9563
HDL	LRL	23	31	0.051	35	20	0.1	19	33	0.499
	Normal	28	41	0.8213	42	27	0.7518	21	48	0.4799
Leptin	Normal	28	31	1.422	37	22	0.239	19	40	0.104
	URL	22	38	0.2331	35	25	0.6249	21	39	0.7471
rs7799039 of *LEP* gene	GG + GA	43	66	2.773	66	43	0.032	36	73	1.064
	AA	5	2	0.0959	4	3	0.858	1	6	0.3023
rs7799039 of *LEP* gene	AA + GA	33	60	**4.131**	55	39	0.697	29	65	0.249
	GG	13	9	**0.0421**	15	7	0.4038	8	14	0.6178
rs2167270 of *LEP* gene	GG + GA	39	60	0.019	60	39	0.019	32	67	0.057
	AA	7	10	0.8904	10	7	0.8904	5	12	0.8113
rs2167270 of *LEP* gene	AA + GA	29	41	0.232	42	28	0.009	25	45	1.184
	GG	17	29	0.63	28	18	0.9244	12	34	0.2765
rs16138 of *NPY* gene	GC	22	34	0.006	34	22	0.006	17	39	0.118
	GG	24	36	0.9383	36	24	0.9383	20	40	0.7312
rs16478 of *NPY* gene	GA	23	38	0.204	35	26	0.474	18	43	0.338
	GG	23	32	0.6515	35	20	0.4912	19	36	0.561

95% CI: 7.5–23.8) compared to patients with normal weight and overweight (13 months, 95% CI: 8.5–17). Lipid profile, leptin concentration, and *LEP* and *NPY* gene polymorphisms did not affect median PFS and OS and risk of progression or death (Table 3).

Multivariate analysis (all factors listed in Table 3) was performed also by multivariate Cox proportional-hazards regression. The only factor significantly affecting the risk of progression was the expression of PD-L1 on TC. Patients with PD-L1 expression on ≥50% of tumor cells had a lower risk of progression than patients with TPS<50% (HR=0.6068, 95% CI: 0.4001–0.9204, $p=0.0187$). The only factor that slightly affected the risk of death was leptin concentration. The risk of death was reduced in patients with high leptin levels compared to

Table 3 Progression free survival and overall survival in patients received immunotherapy according to demographic factors, clinical factors and lipid profile. Abbreviation: mPFS – median progression free survival, mOS – median overall survival, SCC – squamous cell carcinoma, TC – tumor cells, UPR – upper reference limit, LRL – lower reference limit, *LEP* – leptin gene, *NPY* – neuropeptide Y gene

Population characteristic		mPFS (months, 95% CI)	HR (95% CI), *p*	mOS (months, 95% CI)	HR (95% CI), *p*
Whole group		5 (3-5.5)	-	12 (8–16)	-
Gender	Female	5.5 (3–11)	0.9272 (0.587–1.4644), 0.7457	13 (8.5–25)	0.9716 (0.5793-1.62-94), 0.913
	Male	4.9 (3-7.5)		13.5 (7.5–18.5)	
Age	< 65 years	5.5 (2.5–7.5)	1.1833 (0.7372–1.8993), 0.4857	18 (7.5–30.5)	0.7328 (0.434–1.2372), 0.2447
	≥ 65 years	5 (3–9)		13 (8.5–17)	
Diagnosis	Non-SCC	5 (3–9)	0.9813 (0.6267-1.53-65), 0.9343	16.5 (7.5–23.5)	0.9366 (0.5639–1.5557), 0.8004
	SCC	5 (3–8)		13 (8.5–18.5)	
Stage of disase	IIIB	2.8 (2-5.5)	1.2407 (0.5225–2.9462), 0.6249	8.5 (4-16.5)	1.385 (0.5316–3.6087), 0.505
	IV	5.5 (4–8)		13.5 (9.5–23)	
PD-L1 expression	< 1% of TC	3 (2.5-5)	1.4948 (0.792–2.8214), 0.2148	9.5 (5–18)	1.266 (0.6528–2.4551), 0.4852
	≥ 1% of TC	5.5 (4-8.5)		16 (8.5–23.5)	
PD-L1 expression	< 50% of TC	**4 (2.55–5.5)**	**1.5406 (0.9585–2.4761), 0.0743**	13.5 (8.5–18)	1.2416 (0.7401–2.0829), 0.4122
	≥ 50% of TC	**6.5 (4–12)**		13 (7.5–30.5)	
Line of treatment	First line	5.5 (3-11.5)	0.8334 (0.5288–1.3136), 0.4324	13 (7.5–23)	1.1857 (0.7052–1.9936), 0.5206
	Second line	5 (3-6.5)		13.5 (8.5–18)	
Obesity	No	**4.3 (3-6.5)**	**1.2161 (0.7207–2.0519), 0.4636**	**13 (8.5–17)**	**1.4144 (0.7834–2.5536), 0.2501**
	Yes	**6.6 (3–12)**		**23.8 (7.5–23.8)**	
Cholesterol	Normal	5 (3-6.6)	1.0740 (0.6619–1.7426), 0.7725	13.5 (8-23.5)	0.9688 (0.5581–1.6818), 0.9104
	URL	5 (2.5–11)		13.5 (8.5–23)	
Triglyceride	Normal	5 (4-7.5)	0.9709 (0.5979–1.5765), 0.905	13 (8.5–18.5)	1.1171 (0.6525–1.9125), 0.6864
	URL	4 (2–11)		16.5 (7–27)	
LDL	Normal	5 (3-7.5)	1.1159 (0.6179–2.0154), 0.7162	13.5 (8.5–18.5)	1.1145 (0.576–2.1562), 0.7475
	URL	4 (2.5–11)		17 (5–23)	
Non-HDL	Normal	4.3 (3-6.5)	1.1576 (0.7336-1.82-66), 0.5294	13.5 (7.5–23.5)	1.1415 (0.6818–1.9113), 0.6147
	URL	5.5 (3.5–9.5)		16.5 (9.5–27)	
HDL	LRL	4 (2.6–8.5)	1.2698 (0.8086–1.9939), 0.2996	13.5 (7–23)	1.2533 (0.7516–2.0902), 0.3868
	Normal	5 (4–8)		16.5 (9.5–25)	
Leptin	Normal	4 (3–9)	1.2334 (0.7824–1.9446), 0.3664	13 (7.5–17)	1.1864 (0.7061–1.9934), 0.5184
	URL	5.5 (4.3–8.5)		18 (8.5–25)	
rs7799039 of *LEP* gene	GG + GA	5 (4-7.5)	0.9058 (0.3725–2.2019), 0.8272	16 (8.5–23.5)	0.8733 (0.329–2.3179), 0.7856
	AA	5.5 (2-17.5)		13.5 (8.5–16.5)	
+rs7799039 of *LEP* gene	AA + GA	5.5 (4-8.5)	0.7674 (0.4161–1.4155), 0.3967	16 (9.5–23)	0.8178 (0.402–1.664), 0.5789
	GG	4 (2.5–12)		9.5 (6-10.5)	
rs2167270 of *LEP* gene	GG + GA	5.5 (3–8)	0.9965 (0.501–1.9821), 0.992	16.5 (9.5–23.5)	0.6393 (0.2783–1.4686), 0.2917
	AA	5 (3-8.5)		8 (5.1–13.5)	
rs2167270 of *LEP* gene	AA + GA	5 (3-8.5)	1.0231 (0.6426–1.6292), 0.9232	11.5 (7–23)	1.1197 (0.6608–1.8971), 0.6744
	GG	5.5 (3-9.5)		16 (9.5–23)	
rs16138 of *NPY* gene	GC	4.9 (3-15.5)	0.8059 (0.5071–1.2809), 0.3614	16.5 (7.4–23.5)	1.0199 (0.6037–1.7231), 0.9412
	GG	5.5 (3-7.5)		13.5 (8.5–23.8)	
rs16478 of *NPY* gene	GA	5.5 (3-15.5)	0.7667 (0.4805–1.2233), 0.2651	13.5 (7.5–23.5)	1.0094 (0.5965–1.708), 0.9722
	GG	5 (3-6.5)		13.5 (8.5–23.8)	

those with low protein concentrations (HR = 0.6743, 95% CI: 0.4243-1-0715, $p = 0.0953$).

The analysis of OS and PFS was also performed in subgroups of patients who differed the treatment method. First, we analyzed the impact of the examined factors on the effectiveness of immunotherapy in monotherapy (after excluding patients treated with chemoimmunotherapy). Secondly, we compared the impact of the studied factors on the effectiveness of immunotherapy in monotherapy separately in the first and second lines of treatment. In none of these three subgroups, the lipid profile, presence of obesity, *LEP* and *NPY* genes polymorphism did not affect the effectiveness of immunotherapy measured by the percentage of patients with disease control, 6-months PFS and 6-months OS as well as the risk of progression and death.

Discussion

Lipid profile, obesity and leptin concentration may affect the effectiveness of immunotherapy by influencing the expression of PD-L1 on cancer cells and the activity of immune cells. A clear negative relationship exists between TPS and total cholesterol, LDL and non-HDL cholesterol levels. However, a negative effect of high levels of these cholesterol fractions on progression-free survival and overall survival in NSCLC patients receiving immunotherapy could not be demonstrated. This is probably due to our study group's small number of patients and its heterogeneity. Obesity and the associated high leptin concentration have a slight beneficial effect on the chance of achieving disease stabilization and prolongation of overall survival. The only studied polymorphism that may affect the effectiveness of immunotherapy and prognosis is rs7799039 in the *LEP* gene. Compared to patients with the GG genotype, patients with the AA or GA genotype had a significantly lower TPS. In contrast, the rate of patients with disease control was higher in carriers of the AA or GA genotype than in patients with the GG genotype. Considering these limitations of our study, the planning of future experiments should focus on patients treated with first-line immunotherapy in monotherapy to elucidate the actual impact of the lipid profile and obesity as well as leptin concentration and *LEP* gene polymorphisms on the effectiveness of this method of treatment or the prognosis of patients with advanced NSCLC. One notable factor influencing the results was the unexpectedly high rate of complications in the chemoimmunotherapy cohort, leading to the premature discontinuation of treatment in 7 patients. This issue may be attributed to the relatively small sample size of patients receiving chemoimmunotherapy (21 individuals) and potentially unfavorable circumstances. These patients were referred for alternative therapies or transferred to other medical centers, resulting in incomplete observational data. Consequently, the immature data from these patients direct impact the observed overall survival, contributing to the reduced OS reported in this study cohort.

Eating disorders and their impact on the immune system and cancer risk

Cholesterol has the greatest impact on immune system activation and therefore may influence the cancer course and the effectiveness of immunotherapy. Cholesterol is involved in the remodeling the TME and tertiary lymphoid structures (TLSs) [28–31]. Cholesterol is vital in the activation, migration and immunity of T and B lymphocytes and increases the number of monocytes and neutrophils. It induces inflammatory activity in macrophages, which is significant in the context of cancer and immunotherapy [4, 32, 33] In NSCLC patients, statins (cholesterol-lowering drugs), reduce the number of tumor-associated macrophages (TAMs). Metabolism-targeted drugs can enhance cancer immunotherapy by modulating the immunosuppressive tumor microenvironment (TME). Administration of a FATP2 inhibitor (a fatty acid transport protein crucial in lipid metabolism) in combination with PD-1 or CTLA-4 inhibitors increased CD8+T cell infiltration in the TME and reduced tumor progression. Liver X receptors (LXRs) are a family of nuclear receptors that play a crucial role in regulating lipid, cholesterol, and glucose metabolism. In the preclinical studies, the LXR agonist – RGX-104 induced regression of several tumors by reducing the number of myeloid-derived suppressor cells (MDSCs) in tumors. The combination of RGX-104 with anti-PD-1 enhanced antitumor activity in lung cancer and melanoma [4]. Furthermore, a triple therapy involving adoptive T cells, an anti-PD-1 antibody, and an LXR agonist significantly increased antitumor activity.

Patients with low HDL levels had were observed to have a higher percentage of NK cells than those with high HDL levels [28]. Low LDL levels were associated with a worse anti-cancer response. It was discovered that cholesterol establishes PD-1 expression in immune cells [28]. Patients with low LDL compared to patients with normal LDL had a higher ratio of PD-1+T cells to CD20+B cells in TLSs, a higher ratio of PD-1+T cells to CD8+T cells, and an increased percentage of PD-1+T cells in the extra-TLS zone.

There is an apparent relationship between obesity and an increased risk of cancer [34, 35]. However, the significance of obesity is different in patients with already developed cancer. Numerous studies indicate that in these patients, obesity may be a fovurable prognostic factor in particular types of cancer.

Meta-analysis performed by Liu et al. included 31 publications, 6,589,383 people and 62,246 lung cancer cases.

Authors indicated that body-mass index and high-density-lipoprotein cholesterol concentration were negatively correlated with lung cancer risk [36]. It is essential that each of the following: MetS, total cholesterol concentration, triglycerides concentration, low-density lipoprotein cholesterol concentrations and obesity were not associated with lung cancer risk [15]. Authors underlined that the most significantimpact on the development of lung cancer was insulin resistance and type 2 diabetes.

Obesity was a protective factor for shortened overall survival in patients with gastrointestinal tumors (HR=0.67) and lung cancer (HR=0.67) in comparison with patients with normal body weight [34].

It was also discovered that leptin can exert oncogenic or antitumor effects depending on the miRNA-mediated target gene function modulated by adipokines [15, 37]. Apart from the molecular factors, appropriate conditions must also be provided to promote or inhibit cancer such as apoptosis or angiogenesis [15].

Obesity, Weight disorders, leptin levels and the effectiveness of immunotherapy

The main challenge in immunotherapy is to create such conditions for the treatment to obtain the greatest possible immune response against cancer cells [38]. A phenomenon highlighted in numerous reports assessing the effectiveness of immunotherapy in lung cancer patients is the "obesity paradox", where both obesity and overweight are associated with unexpectedly longer survival [15, 39–43]. The paradoxical effect of obesity on cancer may be related to the pro-inflammatory effect of leptin, which is crucial for stimulating the immune system [44]. Increased expression of the genes encoding leptin and leptin receptors has been observed in the tissues of the most common types of cancer.

Obese patients with metastatic NSCLC and TPS≥50% treated with immunotherapy showed an increased objective response rate (OR=1.61, 95% CI: 1.04–2.50), lower risk of progression (HR=0.61, 95% CI: 0.45–0.82) and lower risk of death (HR 0.70, 95% CI 0.49–0.99) compared to patients with normal BMI [45]. Treatment with PD-1/PD-L1 inhibitors activates a survival advantage in obesity [45]. BMI≥30 kg/m^2 was a factor that significantly improved the survival of NSCLC patients treated with atezolizumab [40]. Natably, the benefit was obtained only in patients undergoing immunotherapy, while no benefit was observed in the group of patients who received chemotherapy (docetaxel). Therefore, it is less likely that the longer lifespan of obese patients was only associated with a lower tendency to cachexia. It is assumed that the treatment's greater effectiveness resulted from the stimulation of the immune system by obesity, which mainly led to the activation of T lymphocytes [13, 46]. The existence of a linear association between increasing BMI and decreased risk of death among NSCLC patients treated with various ICIs was indicated, and additionally, a 25% lower risk of progression in obese patients was observed (HR=0.75; 95% CI: 0.62–0.92; p=0.005) [41].

However, some studies found no significant difference in median PFS and OS between overweight or obese NSCLC patients and patients with normal body weight treated with ICIs [47, 48]. It was also emphasized that in overweight and obese cancer patients the time to obtain the best radiographic response was longer in comparison with patients with normal body weight (3.7 months vs. 2.5 months) as well as progression was more common in overweight or obese patients [47]. It should be noted that this group of patients included NSCLC patients treated with immunotherapy or immunotherapy in combination with chemotherapy.

Besides the significant association between overweight and obesity (BMI≥25 kg/m^2) and longer survival in NSCLC patients who received immunotherapy (p<0.01), the importance of other factors affecting the treatment results was underlined, such as weight loss or reduced skeletal muscle mass [13]. If these factors were taken into account, the significance of a high BMI decreased. However, other authors did not confirm the relevance of reduced skeletal muscle mass or percentage of subcutaneous or visceral fat on immunotherapy effectiveness [43, 44]Stillit has been shown that the ratio of leptin concentration to the amount of visceral fat tissue may be necessary, which has been associated with the prolongation of PFS [17].

It should also be highlighted that the results differ depending on the chosen measurement method and the analyzed data. One meta-analysis showed no significant effect of high or low BMI on PFS and OS of NSCLC patients treated with ICIs. However, in this pooled analysis, overweight and obese patients achieved a significant reduction in risk of progression or death compared to patients with normal-weight (HR=0.862; 95% CI: 0.760–0.978, p=0.021 for progression risk and HR=0.818; 95% CI: 0.741–0.902, p<0.0001 for death risk) [48].

Improved anti-tumor response of ICIs was obtained not only in humans but also in obese animals. It allowed further experiments to be carried out. Based on the above data, the experiment on mice was conducted. Low-weight mice were treated with leptin and tumors were measured over time. Leptin treatment, acute or chronic, increased anti-tumor responses like anti-PD-1 therapy [49]. The authors of the experiment explain that simultaneous treatment with anti-PD-1 and leptin may enhance the anticancer effect by increasing the engagement of type M1 tumor-associated macrophages (TAMs) compared to mice not treated with leptin. Studies on diet-induced obese mice (DIO) have brought very interesting

conclusions, in which the duality of obesity in cancer is visible.

On the one hand, DIO mice, compared to non-obese mice, showed significant increases in the number of memory T cells and PD-1 expression but on the other hand, significantly increased number of dysfunctional, exhausted T cells in the blood, and reduced T cell proliferative capacity was observed, which could have caused the tumor to grow faster [13]. The picture has changed after using immunotherapy. Treatment with anti-PD-1 had no significant effect on the antitumor response in normal-weight mice. Stillin obese mice, it led to a surprisingly significant reduction in tumor mass, inhibition of metastases, and extension of lifespan [13]. In DIO mice, anti-PD-1 therapy was found to inhibit T cell exhaustion and a significant increase in tumor-infiltrating lymphocytes (TILs) in the tumor microenvironment.

The role of lipid metabolism disorders in carcinogenesis and the effectiveness of immunotherapy in cancer patients is controversial. It seems that higher serum lipid levels correlate with improved treatment outcomes in patients with non-small cell lung cancer undergoing immunotherapy. Moreover, a decrease in specific cholesterol fractions can identify tumors more likely to respond to immunotherapy [50]. Low levels of triglycerides and total cholesterol as well as high-density lipoprotein cholesterol before treatment were associated with a higher risk of death and higher risk of progression in patients receiving ICIs. Low levels of LDL were linked to a higher risk of death. The positive impact of lipids was observed in NSCLC patients treated with immune checkpoint inhibitors in combination with radiotherapy, indicating that low levels of triglycerides, total cholesterol, and LDL were significantly more common in patients with short overall survival In contrast low HDL levels were a prognostic factor for shorter PFS. Radiotherapy may enhance the effectiveness of immunotherapy through its effects on lipid metabolism and may become another crucial treatment element.

Additionally, a higher number of mutations in the lipid metabolism pathway was associated with positive prognosis in patients treated with ICIsPatients with a high number of mutations also showed higher expression of immune checkpoints such as PD-L1, PD-1, B7-H3, and LAG3. These findings suggest that a high mutation rate in the lipid metabolism pathway, combined with high TMB, may serve as a predictive marker for better prognosis in checkpoint inhibitor therapy [12].

It is also worth mentioning a study that analyzed the correlation between lipid changes and treatment outcomes in patients with advanced NSCLC treated with PD-1/PD-L1 inhibitors combined with anlotinib (an anti-angiogenic drug) [51]. The lipid composition was assessed before and after therapy. Nosignificant changes in lipid levels post-treatment were observed in patients with partial response. In the stable disease group, an increase in one phosphatidylglycerol (PG) and three phosphatidylinositols (PI) was noted post-treatment. In the progressive disease group. A significant increase in two PG and 17 PI was observed post-therapy in the progressive disease group. These results suggest that lipid homeostasis is crucial for the efficacy of the combination of PD-1/PD-L1 inhibitors and anlotinib [51].

In our study, we indicated the potential beneficial role of obesity and high leptin concentration in lung cancer patients treated with ICIs. Similarly to the other authors, we reported the relation between leptin concentration and disease course. Leptin concentration was significantly higher ($p=0.012$) in patients with stable disease compared to patients with disease progression during immunotherapy. Median PFS was highest in patients with TPS ≥ 50% (6.5 months) and in obese patients (6.6 months). Obese patients also had a slightly longer median OS compared to other patients (23.8 vs. 13 months). Moreover, the only factor reducing the risk of death was high leptin concentration. However, this effect was not statistically significant ($p=0.0953$). Moreover, leptin and lipid metabolism had no effect on overall survival. The above results may contribute to a better understanding of the mechanisms of metabolic regulation in lung cancer and the impact on the effectiveness of treatment.

Genetic polymorphisms of the *LEP* gene and the effectiveness of immunotherapy

Statistical significance was demonstrated regardingthe correlation between the GG genotype of the rs7799039 polymorphism of the *LEP* gene and higher PD-L1 expression. However, disease control occurred significantly more often in patients with the GA or AA genotype (64.5% of patients) compared to patients with the GG genotype (42.9% of patients) in the rs7799039 polymorphism of the *LEP* gene. However, the group of patients with the GG genotype consisted of only 21 patients.

Terrasi et al. suggested that the occurrence of AA or AG genotypes in the *LEP* gene could promote leptin protein expression in breast cancer cells resulting in leptin overexpression in tumor tissue. Macello et al. observed that patients with thyroid cancer who presented the AA genotype of rs7799039 in *the LEP* gene had lower serum levels of leptin than those with the AG genotype. The authors showed also that the individuals with the AG genotype also produced higher serum leptin levels than the subjects with the GG genotype. Moreover, Mohamed et al. found that non-obese individuals had the GG genotype, whereas 46% of obese persons had AA or AG genotypes of the *LEP* gene. This polymorphism also affects lipid metabolism. Some authors indicated that compared to people with the GG genotype, carriers of the AA and

AG genotypes have higher LDL cholesterol and triglycerides concentrations, as well as LDL/HDL ratio [52–54]. These observations may explain why patients with the AA or AG genotype were more likely to benefit from immunotherapy. However, there are also opposite observations, such as those presented by Shetty et al., who examined patients with polycystic ovarian syndrome [55]. The effects of leptin depend on subtle differences in its structure or its receptor [56]. Gene polymorphism involves the replacement of one nucleotide in the DNA structure. In humans, the *LEP* gene is located on the long arm of chromosome 7 (7q31.3) and consists of 3 coding exons and two non-coding introns, composed of approximately 20,000 base pairs. Several polymorphisms have been demonstrated in this gene, including functional polymorphism p.Val110Met, polymorphisms in the promoter region c.-188 C>A and c.-2548 G>A. The most frequently studied polymorphisms of the leptin receptor gene are functional polymorphisms, such as p.Gln223Arg in exon 6 and p.Lys109Arg in exon 4 [57]. In addition, there is a silent T>C variant in codon 343 and a G>A variant in codon 1019 of the gene encoding the leptin receptor. Additionally, several other polymorphisms are known within the leptin receptor gene, located both in the promoter region and outside it.

It should be strongly emphasized that the discussed results come from studies conducted on people of different ethnic origins and with various diseases. The results regarding the influence of *LEP* gene polymorphisms on the effectiveness of immunotherapy in NSCLC patients have yet to be published before, which makes our study very original.

Conclusion

Leptin concentration was significantly higher in obese patients than in patients with normal or low weight and in patients with disease stabilization compared to patients with progression observed during immunotherapy. Disease control occurred significantly more often in patients with the GA or AA genotype compared to patients with the GG genotype in the rs779039 polymorphism of the *LEP* gene. The only factor significantly reducing the risk of progression was TPS≥50%, and the only factor slightly reducing the risk of death was high leptin concentration. Obese patients also had a longer median OS compared to other patients (23.8 vs. 13 months). In none of subgroups, the lipid profile, presence of obesity, *NPY* gene polymorphisms did not affect significantly the effectiveness of immunotherapy measured by the percentage of patients with disease control, 6-months PFS and 6-months OS as well as the risk of progression and death.

Author contributions

MF, AG, PK, and ICh: conceptualized the study and performed the research. MF, BKK, JP, KKK, ICh, PK, and JM collected data, analyzed data, and wrote the original paper. MF, PK, and JM critically reviewed and revised the paper. All authors approved the submitted version.

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Data availability

The data of this study are available from the corresponding authors, upon reasonable request.

Declarations

Consent to participate

All patients gave their written consent to participate in the study. The study was approved by the local Bioethics Committee at the Medical University of Lublin (approval number – KE-0254/95/2018).

Conflict of interest

The authors have declared that no conflict of interest exists.

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References

1. Chen S, Mellan I. Oncology meets immunology: the cancer-immunity cycle. Immunity. 2013;39:1–10.
2. Rangamuwa K, et al. Methods for assessment of the tumour microenvironment and immune interactions in non-small cell lung cancer. A narrative review. Front Oncol. 2023;13:1129195. https://doi.org/10.3389/fonc.2023.1129195.
3. Genova C, et al. Therapeutic implications of tumor microenvironment in lung cancer: focus on immune checkpoint blockade. Front Immunol. 2021;12:799455. https://doi.org/10.3389/fimmu.2021.799455.
4. Bleve A, Durante B, Sica A, Consonni FM. Lipid metabolism and cancer immunotherapy: immunosuppressive myeloid cells at the crossroad. Int J Mol Sci. 2020;21:5845. https://doi.org/10.3390/ijms21165845.
5. Cancer Today. Globocan 2022 (version 1.1) – 08.02.2024. https://gco.iarc.fr/today/en/dataviz/pie?mode=cancer&group_populations=1&sexes=0&types=0.
6. Wang F, Xia T, Li Z, Gao X, Fang X. Current status of clinical trial research and application of immune checkpoint inhibitors for non-small cell lung cancer. Front Oncol. 2023;13:1213297. https://doi.org/10.3389/fonc.2023.1213297.
7. Lee SM, et al. First-line atezolizumab monotherapy versus single-agent chemotherapy in patients with non-small-cell lung cancer ineligible for treatment with a platinum-containing regimen (IPSOS): a phase 3, global, multicentre, open-label, randomised controlled study. Lancet. 2023;402:451–63. https://doi.org/10.1016/S0140-6736(23)00774-2.
8. Cortellini A, et al. Differential prognostic effect of systemic inflammation in patients with non-small cell lung cancer treated with immunotherapy or chemotherapy: a post hoc analysis of the phase 3 OAK trial. Cancer. 2022;128:3067–79. https://doi.org/10.1002/cncr.34348.
9. Chen M, et al. Comparison of chemotherapy plus pembrolizumab vs. chemotherapy alone in EGFR-mutant non-small-cell lung cancer patients. Clin Lung Cancer. 2023;24:278–86. https://doi.org/10.1016/j.cllc.2022.12.003.

10. Rounis K, et al. Cancer cachexia and antitumor immunity: common mediators and potential targets for new therapies. Life (Basel). 2022;12:880. https://doi.org/10.3390/life12060880.
11. Potapov I, Kanneganti TD, del Sol A. Fostering experimental and computational synergy to modulate hyperinflammation. Trends Immunol. 2022;43:78–92. https://doi.org/10.1016/j.it.2021.11.004.
12. Cheng T, Zhang J, Liu D, Lai G, Wen X. Prognosis of non-small-cell lung cancer patients with lipid metabolism pathway alternations to immunotherapy. Front Genet. 2021;12:646362. https://doi.org/10.3389/fgene.2021.646362.
13. Wang Z, et al. Paradoxical effects of obesity on T cell function during tumor progression and PD-1 checkpoint blockade. Nat Med. 2019;25:141–51. https://doi.org/10.1038/s41591-018-0221-5.
14. Pereira S, Cline DL, Glavas MM, Covey SD, Kieffer TJ. Tissue-specific effects of leptin on glucose and lipid metabolism. Endocr Rev. 2021;42:1–28. https://doi.org/10.1210/endrev/bnaa027.
15. Kim JW, Kim JH, Lee YJ. The role of adipokines in tumor progression and its association with obesity. Biomedicines. 2024;12:97. https://doi.org/10.3390/biomedicines12010097.
16. Harris RB. Direct and indirect effects of leptin on adipocyte metabolism. Biochim Biophys Acta. 2014;1842:414–23. https://doi.org/10.1016/j.bbadis.2013.05.009.
17. Vita E, et al. Leptin-mediated meta-inflammation may provide survival benefit in patients receiving maintenance immunotherapy for extensive-stage small cell lung cancer (ES-SCLC). Cancer Immunol Immunother. 2023;72:3803–12. https://doi.org/10.1007/s00262-023-03533-0.
18. Jais A, Brüning JC. Arcuate nucleus-dependent regulation of metabolism-pathways to obesity and diabetes mellitus. Endocr Rev. 2022;43:314–28. https://doi.org/10.1210/endrev/bnab025.
19. Abella V, et al. Leptin in the interplay of inflammation, metabolism and immune system disorders. Nat Rev Rheumatol. 2017;13:100–9. https://doi.org/10.1038/nrrheum.2016.209.
20. Jia Z, Liu Y, Cui S. Adiponectin induces breast cancer cell migration and growth factor expression. Cell Biochem Biophys. 2014;70:1239–45. https://doi.org/10.1007/s12013-014-0047-9.
21. Ray A, Cleary MP. The potential role of leptin in tumor invasion and metastasis. Cytokine Growth Factor Rev. 2017;38:80–97. https://doi.org/10.1016/j.cytogfr.2017.11.002.
22. Kloting N, Bluher M. Adipocyte dysfunction, inflammation and metabolic syndrome. Rev Endocr Metab Disord. 2014;15:277–87. https://doi.org/10.1007/s11154-014-9301-0.
23. Wrann CD, et al. Short-term and long-term leptin exposure differentially affect human natural killer cell immune functions. Am J Physiol Endocrinol Metab. 2012;302:E108–16. https://doi.org/10.1152/ajpendo.00057.2011.
24. Tsiotra PC, Boutati E, Dimitriadis G, Raptis SA. High insulin and leptin increase resistin and inflammatory cytokine production from human mononuclear cells. *Biomed Res. Int* 2013, 487081 (2013). https://doi.org/10.1155/2013/487081
25. Pérez-Pérez A, Sánchez-Jiménez F, Vilariño-García T, Sánchez-Margalet V. Role of leptin in inflammation and vice versa. Int J Mol Sci. 2020;21:5887. https://doi.org/10.3390/ijms21165887.
26. Afrin S, Ramaiyer M, Begum UAM, Borahay MA. Adipocyte and adipokines promote a uterine leiomyoma friendly microenvironment. Nutrients. 2023;15:715. https://doi.org/10.3390/nu15030715.
27. Garofalo C, Surmacz E. Leptin and cancer. J Cell Physiol. 2006;207:12–22. https://doi.org/10.1002/jcp.20472.
28. Zou Y, Yu X, Zhou C, Zhu C, Yuan Y. Adverse effects of low serum lipoprotein cholesterol on the immune microenvironment in gastric cancer: a case-control study. Lipids Health Dis. 2022;21:150. https://doi.org/10.1186/s12944-022-01766-z.
29. Munoz-Erazo L, Rhodes JL, Marion VC, Kemp RA. Tertiary lymphoid structures in cancer - considerations for patient prognosis. Cell Mol Immunol. 2020;17:570–5. https://doi.org/10.1038/s41423-020-0457-0.
30. Cheng N, et al. Prognostic value of tumor-infiltrating lymphocytes and tertiary lymphoid structures in Epstein-Barr virus-associated and -negative gastric carcinoma. Front Immunol. 2021;12:692859. https://doi.org/10.3389/fimmu.2021.692859.
31. Li Q, et al. CD8(+) T cells located in tertiary lymphoid structures are associated with improved prognosis in patients with gastric cancer. Oncol Lett. 2020;20:2655–64. https://doi.org/10.3892/ol.2020.11828.
32. Hao M, et al. Combination of metabolic intervention and T cell therapy enhances solid tumor immunotherapy. Sci Transl Med. 2020;12:eaaz6667. https://doi.org/10.1126/scitranslmed.aaz6667.
33. Liu C, et al. Oxysterols direct B-cell migration through EBI2. Nature. 2011;475:519–23. https://doi.org/10.1038/nature10226.
34. Wen H, et al. Body mass index, weight change, and cancer prognosis: a meta-analysis and systematic review of 73 cohort studies. ESMO Open. 2024;9:102241. https://doi.org/10.1016/j.esmoop.2024.102241.
35. Scherübl H. Metabolic syndrome and cancer risk. Dtsch Med Wochenschr. 2022;147:1068–77. https://doi.org/10.1055/a-1868-9164.
36. Liu J, Wang R, Tan S, Zhao X, Hou A. Association between insulin resistance, metabolic syndrome and its components and lung cancer: a systematic review and meta-analysis. Diabetol Metab Syndr. 2024;16:63. https://doi.org/10.1186/s13098-024-01308-w.
37. Jasinski-Bergner S, Kielstein H. Adipokines regulate the expression of tumor-relevant microRNAs. Obes Facts. 2019;12:211–25. https://doi.org/10.1159/000496625.
38. Tomaszewski W, Sanchez-Perez L, Gajewski TF. Brain tumor microenvironment and host state: implications for immunotherapy. Clin Cancer Res. 2019;25:4202–10. https://doi.org/10.1158/1078-0432.CCR-18-1627.
39. Murphy WJ, Longo DL. The surprisingly positive association between obesity and cancer immunotherapy efficacy. JAMA. 2019;321:1247–8. https://doi.org/10.1001/jama.2019.0463.
40. Kichenadasse G, et al. Association between body mass index and overall survival with immune checkpoint inhibitor therapy for advanced non-small cell lung cancer. JAMA Oncol. 2020;6:512–8. https://doi.org/10.1001/jamaoncol.2019.5241.
41. Lee JH, et al. Obesity paradox in patients with non-small cell lung cancer undergoing immune checkpoint inhibitor therapy. J Cachexia Sarcopenia Muscle. 2023;14:2898–907. https://doi.org/10.1002/jcsm.13367.
42. Guo H, et al. Prognostic value of obesity in patients with cancer treated with immune checkpoint inhibitors: an updated meta-analysis and systematic review. Mol Clin Oncol. 2024;20:5. https://doi.org/10.3892/mco.2023.2703.
43. Rocco D, et al. Prognostic factors in advanced non-small cell lung cancer patients treated with immunotherapy. Cancers. 2023;15:4684. https://doi.org/10.3390/cancers15194684.
44. Sánchez-Jiménez F, Pérez-Pérez A, de la Cruz-Merino L, Sánchez-Margalet V. Obesity and breast cancer: role of leptin. Front Oncol. 2019;9:596. https://doi.org/10.3389/fonc.2019.00596.
45. Hahn AW, Venkatesh N, Msaoeul P, McQuade JL. The influence of obesity on outcomes with immune checkpoint blockade: clinical evidence and potential biological mechanisms. Cells. 2023;12:2551. https://doi.org/10.3390/cells12212551.
46. Zhang T, Li S, Chang J, Qin Y, Ii C. Impact of BMI on the survival outcomes of non-small cell lung cancer patients treated with immune checkpoint inhibitors: a meta-analysis. BMC Cancer. 2023;23:1023. https://doi.org/10.1186/s12885-023-11512-y.
47. Palmer JP, et al. Overweight or obese patients may take longer to respond and be less responsive to immune checkpoint inhibitors in non-small cell lung cancer: a retrospective review. J Clin Oncol. 2021;39:e21209. https://doi.org/10.1200/JCO.2021.39.15_suppl.e21209.
48. Antoun S, et al. Protective effect of obesity on survival in cancers treated with immunotherapy vanishes when controlling for type of cancer, weight loss and reduced skeletal muscle. Eur J Cancer. 2023;178:49–59. https://doi.org/10.1016/j.ejca.2022.10.013.
49. Dudzinski SO, et al. Leptin augments antitumor immunity in obesity by repolarizing tumor-associated macrophages. J Immunol. 2021;207:3122–30. https://doi.org/10.4049/jimmunol.2001152.
50. Zhang J, et al. The baseline serum lipid levels and outcomes of NSCLC patients receiving immunotherapy combined of non-combined with radiotherapy: a single center retrospective study. Int J Radiat Oncol. 2023;117:e11.
51. Liu L, et al. Lipid alterations play a role in the integration of PD-1/PD-L1 inhibitors and anlotinib for the treatment of advanced non-small-cell lung cancer. Lipids Health Dis. 2024;23:16. https://doi.org/10.1186/s12944-023-01960-7.
52. Terrasi M, et al. Functional analysis of the – 2548G/A leptin gene polymorphism in breast cancer cells. Int J Cancer. 2009;125:1038–44. https://doi.org/10.1002/ijc.24372.
53. Marcello MA et al. Polymorphism in LEP and LEPR may modify leptin levels and represent risk factors for thyroid cancer. *Int. J. Endocrinol* 2015, 73218 (2015). https://doi.org/10.1155/2015/173218
54. Mohamed AA, et al. The impact of LEP rs7799039 polymorphism and obesity on the severity of coronavirus disease-19. Diabetes Metab Syndr Obes. 2023;16:515–22. https://doi.org/10.2147/DMSO.S391869.
55. Shetty SS, Kumari N, Hegde P, Roopashree PG, Suhasini PC. Leptin gene polymorphism rs7799039; G2548A, metabolic and oxidative stress markers in

polycystic ovarian syndrome. J King Saud Univ Sci. 2022;34:102222. https://doi.org/10.1016/j.jksus.2022.102222.
56. Guo S, Liu M, Wang G, Torroella-Kouri M, Gonzalez-Perez RR. Oncogenic role and therapeutic target of leptin signaling in breast cancer and cancer stem cells. Biochim Biophys Acta Rev Cancer. 2012;1825:207–22. https://doi.org/10.1016/j.bbcan.2012.01.002.
57. Kim SM, et al. Association of leptin receptor polymorphisms Lys109Arg and Gln223Arg with serum leptin profile and bone mineral density in Korean women. Am J Obstet Gynecol. 2008;198:1–8. https://doi.org/10.1016/j.ajog.2007.10.799.

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