ORIGINAL ARTICLE

Serum PCSK9 levels at the second nivolumab cycle predict overall survival in elderly patients with NSCLC: a pilot study

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Abstract

Monoclonal antibodies targeting PD-1 are used for treating NSCLC. To date, proprotein convertase subtilisin/kexin type 9 (PCSK9) has been poorly investigated in the oncologic field. Here, we aimed at evaluating whether serum PCSK9 might represent a predictive factor for OS in older patients with advanced NSCLC under nivolumab treatment. Among 78 patients with advanced, pre-treated NSCLC previously enrolled in a prospective study at Ospedale Policlinico San Martino in Genoa (Italy), 44 patients have been included in this sub-analysis due to the availability of serum samples for the measurement of PCSK9. Before each nivolumab administration, clinical information and blood samples were collected. Median age was 71, with a prevalence of the male sex. The most represented histological type of lung cancer was adenocarcinoma. The majority of patients were former smokers (72.1%). Median PCSK9 levels were 123.59 (86.32–169.89) ng/mL and 117.17 (80.46–147.79) ng/mL at cycle 1 and 2, respectively. Based on a receiver operating characteristic curve analysis, a PCSK9 value at cycle 2 of 95 ng/mL was found as the best cutoff point for OS. Kaplan–Meier analysis demonstrated that patients below the PCSK9 cutoff (< 95 ng/mL) experienced a better OS, as confirmed by Cox proportional hazard regression analysis. In this pilot study, circulating levels of PCSK9 < 95 ng/mL at the time of the second cycle of nivolumab treatment could independently predict a better OS in elderly patients with advanced, pre-treated NSCLC. However, further studies are warranted to validate these preliminary results.

Abbreviations
AUC   Area under the curve
CI   Confidence interval

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ECOG PS	Eastern Cooperative Oncology Group scale of Performance Status
HR	Hazard ratio
KO	Knock-out
LRP-1	Lipoprotein receptor-related protein-1
NLR	Neutrophil-to-lymphocyte ratio
PC	Proprotein convertase
PCSK9	Proprotein convertase subtilisin/kexin type 9
PLR	Platelet-to-lymphocyte ratio
ROC	Receiver operating characteristic
(si)RNA	Small interfering RNA

Introduction

Lung cancer represents a primary cause of disease and death and is often diagnosed among the elderly [1]. The most common type of lung cancer is NSCLC comprising more than 80% of all cases. Monoclonal antibodies targeting PD-1 are now used for NSCLC due to proven efficacy and safety [2]. Nivolumab is a fully human PD-1 immune checkpoint inhibitor approved for patients with advanced NSCLC, which progressed on or after previous chemotherapy [3]. Nivolumab was demonstrated to increase OS compared to docetaxel by reducing the risk of death by 41% and this trend prolonged across a 2-year follow-up period [4, 5].

Proprotein convertase subtilisin/kexin type 9 (PCSK9) was first described in 2003 to play a role in liver regeneration and neuronal differentiation and later recognized to be also implicated in cholesterol homeostasis [6]. In humans, the clinical importance of PCSK9 increased when its gain-of-function mutation was associated with autosomal dominant hypercholesterolemia [7]. More recently, PCSK9 has become a druggable target as PCSK9 inhibitors showed a large benefit in the field of atherosclerosis and cardiovascular diseases [8, 9]. Proprotein convertases (PCs) were associated with cancer since the early 1990s [10] and their expression was found different between normal and tumor cells and tumors with different aggressive patterns. Accordingly, PC1/PC3 and PC2 were described in small cell lung carcinoma [11] as well as in breast, colon, and head and neck cancers. Since matrix metalloproteinases were recognized as a pivotal tumor-associated PC substrate, it became clear that PCs were very important in tumor development and progression determining malignant phenotype and aggressiveness [12]. To date, few data are available concerning the role of PCSK9 in patients with NSCLC.

In this pilot study, we aimed at assessing circulating PCSK9 levels at different time points among older patients with advanced NSCLC under nivolumab therapy and at evaluating whether PCSK9 might represent a predictive factor for OS in these patients.

Materials and methods

Study design and patients

The present is a pilot study enrolling 44 patients with advanced, pre-treated NSCLC from a larger cohort of 78 patients recruited between April 2015 and June 2016 in a prospective study at the Lung Cancer Unit of IRCCS Ospedale Policlinico San Martino in Genoa (Italy) [13]. Only patients for whom PCSK9 levels for the first two cycles of nivolumab were available have been included in the present study. Main inclusion criteria were reported elsewhere [13]. Bristol-Myers Squibb provided nivolumab within the Italian expanded access program in NSCLC. Nivolumab was administered at the dosage of 3 mg/kg every 14 days. The Eastern Cooperative Oncology Group scale of Performance Status (ECOG PS) was used to assess the functional status of a patient [14].

Blood samples were collected at each of the first five scheduled cycles of nivolumab treatment (before drug administration) and both serum and plasma were stored at −80 °C until analysis. Clinical history and examination were retrieved from medical records.

Study endpoints

The primary endpoint was to investigate the predictive role of PCSK9 towards the OS during nivolumab treatment. Secondary endpoints included the measurement of PCSK9 fluctuations during nivolumab therapy and the evaluation of correlations between PCSK9 and clinical parameters.

Detection of biochemical and inflammatory biomarkers

Hematological and biochemistry variables were measured by routine auto-analyzers. Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) were calculated using the following equations: NLR = absolute neutrophil count/absolute lymphocyte count and PLR = absolute platelet count/absolute lymphocyte count. PCSK9 was measured by colorimetric ELISA following the manufacturer's instructions (R&D Systems, Minneapolis, MN). The limits of detection for PCSK9 was 62.5 pg/mL with mean intra- and inter-assay coefficients of variation below 8%, as previously reported [9].

Statistical analysis

Analyses were performed with IBM SPSS Statistics for Windows, Version 23.0 (IBM CO., Armonk, NY), MedCalc 12.5 (MedCalc Software, Ostend, Belgium), and GraphPad Prism

5 (GraphPad Software, Inc, La Jolla, CA, USA). Categorical data are presented as absolute and relative frequencies and compared with Fisher's exact test, while continuous variables are shown as median and interquartile range (IQR) and their comparison was done by non-parametric Mann–Whitney U test. Ranked Spearman's correlation coefficients were computed to establish correlations between PCSK9 and blood cells, biochemical markers, and clinical parameters. The prognostic ability of PCSK9 was evaluated by a receiver operating characteristic (ROC) curve. The area under the curve (AUC) was given with 95% confidence interval (CI) and the cutoff point of PCSK9 was calculated maximizing the sensitivity in accordance with the Youden index. Kaplan–Meier analysis with log rank test was applied to estimate cumulative survival and to calculate the corresponding risk difference according to PCSK9 levels. The effect of PCSK9 on the OS was obtained from Cox proportional hazards models and expressed as hazard ratio (HR) and 95% CI. In the multivariate model, we adjusted for age, sex, smoking habit, and previous treatments. For all statistical analyses, a two-sided p value < 0.05 was considered as statistically significant.

Results

Patients' characteristics

Clinical characteristics of the overall cohort at baseline (before first cycle of nivolumab) are illustrated in Table 1. Median age was 71 (66–77) with a prevalence of the male sex. The most represented histological type of lung cancer was adenocarcinoma (72.1%). The majority of patients were former smokers and shared a performance status between 0 and 1 (36.4% and 59.1%, respectively). PCSK9 levels are shown in Fig. 1. Median PCSK9 level was 123.59 (86.32–169.89) ng/mL at cycle 1 and dropped at cycle 2 (117.17, 80.46–147.79 ng/mL) with a statistical significance between cycle 2 and 5 as well as between cycle 3 and 5 (Fig. 1). The median follow-up period was 263 days. Death occurred in 37 patients.

Serum PCSK9 levels at the second cycle of nivolumab predict overall survival

As depicted in Table 2, PCSK9 did not correlate with any clinical parameter, except at cycle 2 where a positive correlation with the occurrence of death was found ($r = 0.333$, $p = 0.027$). Then, we tested the ability of serum PCSK9 to predict a reduced OS. Based on a ROC curve analysis, serum levels of PCSK9 showed a significant prognostic accuracy for reduced OS during the follow-up period (AUC 0.762 [95% CI 0.610–0.878]; $p = 0.0011$,

Table 1 Baseline characteristics of the overall cohort

	$n = 44$
Age, years (IQR)	71 (66–77)
Males, n (%)	33 (75.0)
Females, n (%)	11 (25.0)
Smoking habit	
Current, n (%)	13 (32.5)
Former, n (%)	23 (57.5)
Never, n (%)	4 (10.0)
Packs/year, n (%)	56 (40–85)
ECOG PS, n (%)	
0, n (%)	16 (36.4)
1, n (%)	26 (59.1)
2, n (%)	2 (4.5)
Liver metastasis, n (%)	10 (23.3)
Histology	
Adenocarcinoma, n (%)	31 (72.0)
Squamous cell, n (%)	10 (23.3)
Other, n (%)	2 (4.7)
Laboratory findings	
Total WBC, $n \times 10^9$/L (IQR)	8.47 (7.05–10.92)
Neutrophils, $n \times 10^9$/L (IQR)	6.49 (4.58–8.23)
Lymphocytes, $n \times 10^9$/L (IQR)	1.30 (0.9–1.81)
Monocytes, $n \times 10^9$/L (IQR)	0.53 (0.4–0.78)
Platelets, $n \times 10^9$/L (IQR)	290 (222.25–331.5)
PLR (IQR)	206.67 (152.58–295.92)
NLR (IQR)	4.76 (2.62–6.67)
Creatinine, mg/dL (IQR)	1 (0.8–1.2)
eGFR, mL/min/1.73 m^2 (IQR)	75 (61–93.5)
Uric acid, mg/dL (IQR)	4.78 (3.84–5.81)
Fibrinogen, g/L (IQR)	4.4 (3.76–5.26)
Albumin, g/L (IQR)	39.5 (36.45–42.97)
Glycemia, mg/dL (IQR)	98 (89–118)
D-dimer, µg/L	1028.5 (453.23–1837.25)
NSE, ng/mL (IQR)	7.6 (6.21–11.18)
CYFRA 21.1, ng/mL (IQR)	4.77 (2.43–12.24)

Data are expressed as median (interquartile range [IQR], number [n], or percentages [%])

ECOG PS Eastern Cooperative Oncology Group scale of Performance Status, *WBC* white blood cells, *PLR* platelet-to-lymphocyte ratio, *NLR* neutrophil-to-lymphocyte ratio, *eGFR* estimated glomerular filtration rate, *NSE* neuron-specific enolase

Fig. 2). In accordance with the Youden index, a serum PCSK9 value at cycle 2 of nivolumab treatment of 95 ng/mL was found as the best cutoff point, with a sensitivity of 78.4% and a specificity of 85.7% (Fig. 2). When comparing patients according to the cutoff value (95 ng/mL), no difference was encountered (Table 3). Accordingly, Kaplan–Meier analysis confirmed that patients above PCSK9 cutoff experienced a reduced OS across the follow-up period (Fig. 3). Of the 37 events, eight occurred
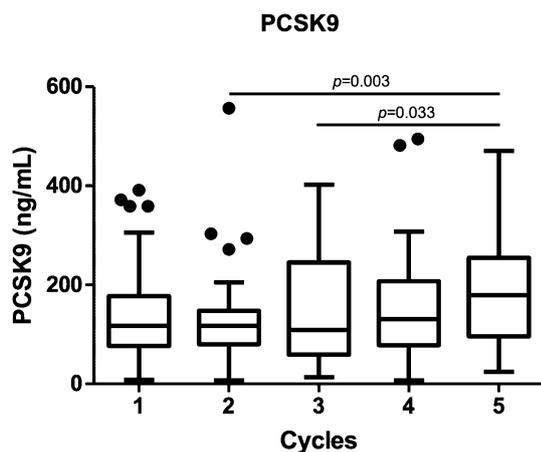
PCSK9

Fig. 1 Circulating levels of PCSK9 across the five cycles of nivolumab treatment included in the study

in patients with PCSK9 values ≤ 95 ng/mL ($n = 14$) and 29 in those with PCSK9 value > 95 ng/mL ($n = 30$). Finally, the Cox proportional hazard regression analysis confirmed the oft-mentioned results (Table 4). Indeed, the OS increased by 70% for patients with PCSK9 < 95 ng/mL (HR 0.30 [95% CI 0.11–0.79]; $p = 0.009$) in the univariate model and was confirmed in the multivariate model when age, sex, smoking habit, and previous treatments were considered as potential confounders (Table 4).

Discussion

The main novelty of this pilot study is represented by the investigation of the predictive value of serum PCSK9 at the second cycle of nivolumab therapy towards the OS

during the follow-up period. Indeed, this aspect is of utmost importance when considering that patients addressed to nivolumab treatment are usually those with an advanced NSCLC not responsive to previous therapies. Hence, in light of the results of this pilot study, patients with PCSK9 levels > 95 ng/mL at second nivolumab cycle might display a worse response to immunotherapy and so they should be considered for novel experimental treatments or supportive therapy only.
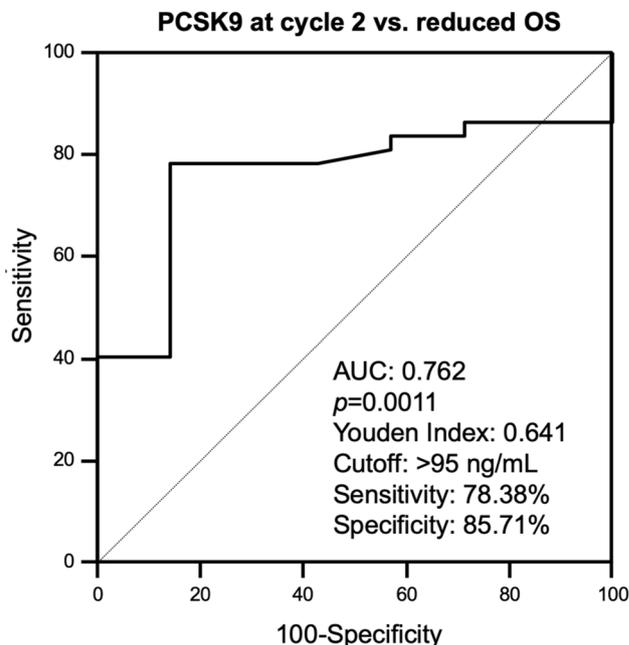

Fig. 2 ROC curve analysis for PCSK9 at cycle 2 toward the reduced OS during the follow-up period

Table 2 Correlations between PCSK9 and blood cells, biochemical markers, and clinical parameters at the time of the first two cycles of nivolumab therapy

Cycle 1			Cycle 2		
PCSK9	r	p		r	p
PCSK9 cycle 2	0.188	0.223	PCSK9 cycle 1	0.188	0.223
Baseline WBC	−0.080	0.608	Baseline WBC	0.051	0.742
Baseline PLT	0.096	0.534	Baseline PLT	−0.025	0.873
Baseline fibrinogen	0.073	0.707	Baseline fibrinogen	0.166	0.389
Baseline glucose	0.073	0.644	Baseline glucose	−0.141	0.367
Baseline albumin	0.066	0.670	Baseline albumin	0.113	0.466
Baseline uric acid	0.113	0.464	Baseline uric acid	0.196	0.203
NSE	−0.408	**0.015**	NSE	−0.056	0.747
CYFRA 21.1	0.002	0.990	CYFRA 21.1	−0.008	0.963
Occurrence of liver metastasis	−0.093	0.552	Occurrence of liver metastasis	−0.242	0.118
Death occurrence	−0.012	0.937	Death occurrence	0.333	**0.027**

Correlations have been performed by Spearman's rank correlation coefficient. Statistically significant correlations are highlighted in bold character

PCSK9 proprotein convertase subtilisin/kexin type 9, *WBC* white blood cells, *PLT* platelets, *NSE* neuron-specific enolase

Table 3 Baseline characteristics of the overall cohort according to PCSK9 cutoff at cycle 2	PCSK9 ≤ 95 ng/mL (n = 14)	PCSK9 > 95 ng/mL (n = 30)	p
Age, years (IQR)	66 (58.5–72.75)	72 (68.5–77.5)	0.062
Males, n (%)	10 (71.4)	23 (76.7)	0.709
Females, n (%)	4 (28.6)	7 (23.3)	
Smoking habit			0.059
Current, n (%)	7 (53.8)	6 (22.2)	
Former, n (%)	4 (30.8)	19 (70.4)	
Never, n (%)	2 (15.4)	2 (7.4)	
Packs/year, n (%)	45 (36–61)	63 (39.5–86.25)	0.334
ECOG PS, n (%)			0.321
0, n (%)	7 (50.0)	9 (30.0)	
1, n (%)	7 (50.0)	19 (63.3)	
2, n (%)	–	2 (6.7)	
3, n (%)	–	–	
Liver metastasis, n (%)	5 (35.7)	5 (17.2)	0.179
Histology			
Adenocarcinoma, n (%)	11 (84.6)	20 (66.6)	0.413
Squamous cell, n (%)	2 (15.4)	8 (26.7)	
Other, n (%)	–	2 (6.7)	
Laboratory findings			
Total WBC, $n \times 10^9$/L (IQR)	7.89 (4.54–10.43)	8.48 (7.60–11.08)	0.236
Neutrophils, $n \times 10^9$/L (IQR)	6.25 (2.68–7.62)	6.49 (4.87–8.43)	0.345
Lymphocytes, $n \times 10^9$/L (IQR)	1.3 (0.88–1.84)	1.3 (0.88–1.85)	0.969
Monocytes, $n \times 10^9$/L (IQR)	0.49 (0.39–0.79)	0.60 (0.41–0.75)	0.444
Platelets, $n \times 10^9$/L (IQR)	289.5 (223–322.75)	290 (220.5–335)	0.982
PLR (IQR)	202.37 (132.57–317.88)	206.67 (160.47–280.23)	0.659
NLR (IQR)	3.47 (1.97–7.06)	4.84 (2.80–6.76)	0.364
Creatinine, mg/dL (IQR)	0.9 (0.70–1.12)	1 (0.8–1.2)	0.423
eGFR, mL/min/1.73 m^2 (IQR)	84 (61.75–97.5)	72 (60.5–86.75)	0.236
Uric acid, mg/dL (IQR)	4.5 (3.02–5.40)	4.85 (4.06–6.42)	0.096
Fibrinogen, g/L (IQR)	4.48 (2.81–5.76)	4.40 (3.86–5.23)	0.875
Albumin, g/L (IQR)	41 (36.85–42.97)	38.9 (35.57–43.07)	0.782
Glycemia, mg/dL (IQR)	98 (94.75–141)	95 (87.5–115.5)	0.294
D-dimer, µg/L	511.3 (424.1–2024.5)	1106 (752.33–1679.25)	0.332
NSE, ng/mL (IQR)	7.08 (6.21–10.63)	7.85 (6.19–11.18)	0.713
CYFRA 21.1, ng/mL (IQR)	3.77 (1.41–8.99)	5.42 (2.45–17.87)	0.308

Data are expressed as median and interquartile range (IQR), number (n), or percentages (%). Statistically significant correlations are highlighted in bold character

PCSK9 proprotein convertase subtilisin/kexin type 9, *ECOG PS* Eastern Cooperative Oncology Group scale of Performance Status, *WBC* white blood cells, *PLR* platelet-to-lymphocyte ratio, *NLR* neutrophil-to-lymphocyte ratio, *eGFR* estimated glomerular filtration rate, *NSE* neuron-specific enolase

PCs were investigated in the oncologic field in the last 30 years and found to play an important role in tumor progression in different systems [12]. Anyway, the role of PCSK9 in these processes was poorly deepened and a very limited number of experimental studies are available. In two studies conducted in humans, a possible link between PCSK9 polymorphisms and the risk of cancer was investigated, but results were controversial [15, 16]. Sun et al. injected B16F1 melanoma cells into both wild-type and PCSK9 knock-out (KO) mice to induce liver metastasis [17].

When on a chow diet, PCSK9 KO mice showed a decreased number of liver metastases as compared to wild-type ones. Anyway, this protection was lost when PCSK9 KO mouse cholesterol levels were normalized by a 2-week high-fat diet. Indeed, a prolongation of the high-fat diet markedly increased metastases in both groups, underlining that high cholesterol levels can promote metastatic progression. Hence, the protective effect of the lack of PCSK9 is not only provided by lower cholesterol levels, but also by the likely enhancement of tumor necrosis factor-alpha-mediated
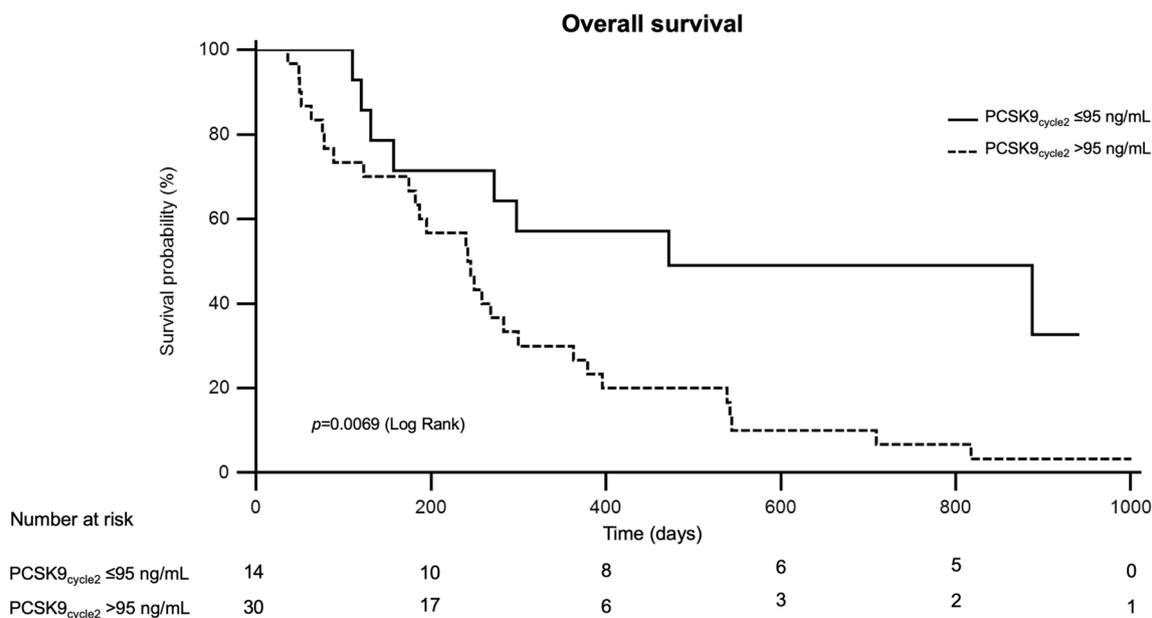
Fig. 3 A PCSK9 value at cycle 2 < 95 ng/mL is associated with an increased OS. Kaplan–Meier survival curve according to high (> 95 ng/mL) and low (≤ 95 ng/mL) PCSK9 levels is shown. The number of events per group is indicated within round brackets

Table 4 Cox proportional hazards model showing the predictive value of PCSK9 cutoff at cycle 2 (≤ 95 ng/mL) towards overall survival during the follow-up period

	Univariate model			Multivariate model		
	HR	95% CI	*p* value	HR	95% CI	*p* value
Overall survival						
Categorized PCSK9 (≤ 95 ng/mL)	0.348	0.156–0.772	**0.009**	0.298	0.113–0.789	**0.015**
Age				0.997	0.949–1.048	0.905
Sex				1.216	0.462–3.199	0.692
Smoking habit				0.895	0.412–1.941	0.778
Previous treatments				1.200	0.844–1.707	0.311

Statistically significant values are highlighted in bold character

HR hazard ratio, *CI* confidence interval, *PCSK9* proprotein convertase subtilisin/kexin type 9

apoptosis [17], suggesting a less comfortable environment for tumor expansion. As a further proof of the role of PCSK9 in tumor development, low-density lipoprotein receptor-related protein-1 (LRP-1) levels were described to be regulated by PCSK9 [18], thus highlighting on the one side LRP-1 as a central actor in tumor metastasis and on the other side PCSK9 as a modulator of the metastatic activity of the tumor. Bhat et al. reported that a decreased expression of PCSK9 and a concurrent increased expression of the receptor of the low-density lipoprotein in hepatocellular carcinoma might suggest that tumor can influence the composition of the local microenvironment to provide a non-stop energy supply [19], thus further confirming an important role of PCSK9 in cancer development. In human

neuroglioma, PCSK9 small interfering (si)RNA was found to promote apoptosis via the activation of caspase-3 and the down-regulation of some anti-apoptotic proteins, whereas PCSK9 overexpression blocked apoptosis [20]. Authors concluded that PCSK9 may have a role in regulating apoptosis through mitochondrial pathway, which can represent a promising therapeutic strategy for the future.

However, a role for PCSK9 has been recently described in lung cancer, too. Accordingly, PCSK9 siRNA has been found to provide an anti-tumor activity via the induction of mitochondrial dysfunction and endoplasmic reticulum-associated cell death in A549 human lung adenocarcinoma cells [21]. To the best of our knowledge, no clinical study has investigated the circulating levels of PCSK9 in NSCLC

patients. In our cohort, we have been able to identify a cut-off value at the time of the second nivolumab cycle, which could predict a reduced OS during the follow-up period. This cutoff value showed both a good sensitivity and specificity and may be worthy to be further tested in larger cohorts and validated.

Some limitations need to be acknowledged when reading our paper. First of all, the small sample size recruited at a single center may limit the generalization of the results, although promising. In fact, a more precise range of PCSK9 levels must be determined as their values show a progressively increasing tendency across cycles other than the second one while the survival rate remains constant. Second, we performed our analysis based only on circulating levels of PCSK9, but no information was available for the expression of this molecule within the lung tissue, so that we could not make any correlation between circulating levels and tissue expression of PCSK9. This aspect definitely deserves to be explored in future studies to establish whether PCSK9 can represent a solid predictive marker of response to immunotherapy and evaluate whether a close relationship between tissue and circulating PCSK9 levels really exists.

In conclusion, circulating levels of PCSK9 > 95 ng/mL at the time of the second cycle of nivolumab treatment can independently predict a reduced OS in older patients with advanced, pre-treated NSCLC. Of importance, the assessment of serum PCSK9 might represent a useful tool for clinicians to evaluate and address patients with advanced NSCLC to the best therapeutic strategy. Starting from this pilot study, future studies are warranted to deepen pathophysiological mechanisms between PCSK9 and cancer development both within lung tissue and in the bloodstream as well as to confirm these preliminary results coming from a limited number of patients.

Compliance with ethical standards

Conflict of interest Francesco Grossi has consulting/advisory relationship with and received honoraria from Bristol-Myers Squibb, Merck Sharp & Dohme, and Pierre Fabre and has consulting/advisory relationship with AstraZeneca and Roche. Carlo Genova received honoraria from AstraZeneca, Boehringer-Ingelheim, Bristol-Myers-Squibb,

Merck Sharp & Dohme, and Roche. Erika Rijavec received honoraria from Bristol-Myers Squibb. Paolo Spallarossa has consulting/advisory relationship with Incyte, Teva, Bristol-Myers Squibb and received honoraria from Incyte, Teva, and Servier. All other authors declare no conflicts of interest.

Ethical approval The study was approved by the Institutional Review Board of IRCCS Ospedale Policlinico San Martino, Genoa, Italy (registry number: P.R. 191REG2015). The study was conducted in accordance with the Declaration of Helsinki and consistent with International Conference on Harmonization (ICH) Guidelines for Good Clinical Practice.

Informed consent Prior to inclusion in the study, all patients gave written, informed consent to the use of their samples and data for research and scientific publications.

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