Baseline Serum Cholesterol Levels Predict the Response of Patients with Advanced Non-Small Cell Lung Cancer to Immune Checkpoint Inhibitor-Based Treatment

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Purpose: Although predictive markers of immune checkpoint inhibitor (ICI)-based treatments have been extensively studied, with the exception of programmed death ligand 1 (PD-L1), most are not widely used in the clinic due to poor effects or defective practicability. The aim of this study was to identify those patients with high baseline serum cholesterol who benefit from ICI-based treatments.

Patients and Methods: Patients with advanced non-small cell lung cancer (NSCLC) treated at Ningbo Medical Center, Li Huili Hospital between August 2017 and December 2019 were enrolled in this retrospective study. The Response Evaluation Criteria in Solid Tumors (RECIST, version 1.1) were used to evaluate the efficacy of the ICI-based treatment. Progression-free survival (PFS) and overall survival (OS) were calculated using the Kaplan–Meier survival curves and compared using the log rank test. Univariate and multivariate analyses were conducted using the logistic regression analysis and Cox proportional hazards model. A receiver operating characteristic curve was created, and the area under the curve (AUC) was calculated to compare the predictive value of baseline serum cholesterol with PD-L1 expression for patient response to ICI-based treatment.

Results: In our cohort of 169 NSCLC patients, the objective response rate (ORR) and disease control rate (DCR) of the treatment were significantly higher in patients with hypercholesterolemia (>5.18 mmol/L) than in those with hypocholesterolemia (ORR: 33.67% vs 14.08%, P=0.004; DCR: 68.37% vs 42.25%, P=0.001). The median PFS was 7.9 months in the hypercholesterolemia group, significantly longer than in the hypocholesterolemia group (4.4 months, 95% CI: 4.620–7.380, P=0.001). The median OS in the two groups were 11 months and 8 months, with 95% CIs of 8.980–10.420 (P<0.001). The AUC for the baseline level of cholesterol was 0.706 (P=0.001), while it was 0.643 (P=0.001) for PD-L1 expression.

Conclusion: The baseline serum cholesterol level is predictive of a clinical benefit for advanced NSCLC patients who undergo ICI-based treatment, and hence it is a promising prognostic indicator for ICI-based treatment of NSCLC.

Keywords: non-small cell lung cancer, NSCLC, serum cholesterol, immune checkpoint inhibitors, ICIs, prognostic marker

Introduction

Lung cancer is associated with high morbidity and mortality throughout the world, with 2,093,876 new cases and 1,761,007 new deaths in 2018.1 Non-small cell lung
cancer (NSCLC) is the dominant subtype. After chemotherapy and targeted therapy, immune-checkpoint inhibitors (ICIs) have offered a new approach to advanced NSCLC. ICIs target the programmed cell death protein 1 (PD-1)/programmed death ligand-1 (PD-L1) axis, and include PD-1 inhibitors like nivolumab and pembrolizumab, and PD-L1 inhibitors like atezolizumab and durvalumab. ICI-based treatment has been standard for NSCLC patients in recent years. Still, the number of patients who benefit from such treatment is small, and predictive biomarkers are urgently needed. Tumor mutation burden, tumor-infiltrating lymphocytes, changes in peripheral blood CD8+ T cells, and immune cell repertoires are potential biomarkers that have been identified in clinical practice.

Metabolic diseases have recently attracted a lot of attention in patients with metastatic cancer. For example, obesity, body mass index (BMI), diabetes, and dyslipidemia were reported to be risk or prognostic factors. Recent studies have also demonstrated that such metabolic conditions are associated with NSCLC patient response to ICI treatment. High serum cholesterol, known as hyperlipidemia, is a type of dyslipidemia. In preclinical studies, high serum cholesterol interacted with immune cells to enhance their anti-tumor properties. Retrospective studies revealed that high serum cholesterol offers positive prognostic value in some types of cancers, including NSCLC, treated with ICIs, and statins have been observed to stimulate immune responses and synergize with anti-PD-1 antibodies in NSCLC.

This study compared clinical outcomes with different levels of serum cholesterol and identified high baseline serum cholesterol levels as being associated with the benefits of ICI-based treatments for NSCLC patients.

Patients and Methods
Patient Eligibility
This study enrolled patients with advanced NSCLC treated at Ningbo Medical Treatment Center, Li Huili Hospital from August 2017 to December 2019. Enrolled patients satisfied the following criteria: (1) diagnosed with histologically or cytologically confirmed NSCLC without mutation of EGFR/ALK/ROS1, (2) presented with measurable lesions, and (3) treated with ICI-based therapy. Treatment was stopped when there were serious infusion-related adverse events, disease progression, or patients refused to continue treatment. Patients’ clinicopathological features were examined. The end of the follow-up period was December 31st 2019.

This study was conducted in accordance with the Declaration of Helsinki. And it was approved by the Ethics Committee of Ningbo Medical Treatment Center, Li Huili Hospital. Written informed consent from patients was waived because of the observational nature.

Definitions of Variables
Patient response to ICI-based treatment was assessed using the Response Evaluation Criteria in Solid Tumors (RECIST, version 1.1). Disease control rate (DCR) was defined as complete or partial response plus stable disease. Progression-free survival (PFS) was defined as the time from the start of ICI-based treatment to confirmed disease progression or death, whichever occurred first. Overall survival (OS) was measured from the date of the first ICI-based treatment to death or the last day of follow-up. If patients were still alive without disease progression before the deadline for analysis or last follow-up date, the data were censored.

PD-L1 expression was detected by the 22C3 antibody, and categorized using TPS (tumor proportion score) cutoffs of 1% and 50%: negative (<1%), low (1–49%), and high (≥50%). There were two types of treatment: ICI-based treatment alone, classified as monotherapy, with nivolumab (3mg/kg q2w), pembrolizumab (200mg q3w), camrelizumab (200mg q3w), and sintilimab (200mg q3w). Or ICIs plus chemotherapy, combined with pemetrexed/paclitaxel plus platinum (cisplatin/carboplatin) based on PD-L1 expression using standard doses and schedules.

We used a value of 5.18 mmol/L total cholesterol (TC) to divide patients into hypercholesterolemia and hypcholesterolemia groups according to American Heart Association Guidelines, with cutoff values of 1.76 mmol/L for triglyceridemia (TG), 3.37 mmol/L for low-density lipoprotein (LDL) cholesterol, and 1.04 mmol/L for high-density lipoprotein (HDL) cholesterol. Fasting serum levels of total cholesterol, triglycerides, LDL cholesterol, and HDL cholesterol were measured before ICI-based treatment. BMI was calculated at the time of treatment initiation, with 25kg/m² serving as the cut-off value. If a patient had a history of statin use, then statin therapy was recorded as “yes”.

Statistical Analysis
SPSS version 22.0 was used for statistical analyses. Pearson’s χ² and independent t-tests were used to make comparisons using patients’ clinicopathological data. Kaplan-Meier was used to estimate PFS and OS, and a Log rank test was used to compare survival rates. Univariate and multivariate analyses for objective response was performed.
Table 1 Baseline Characteristics of Patients (n=169)

| Characteristics                 | All        | Hypcholesterolemia | Hypercholesterolemia | P-value |
|---------------------------------|------------|--------------------|----------------------|---------|
| Age (years)                     |            |                    |                      |         |
| <60                             | 69 (40.83%)| 30 (42.25%)        | 39 (39.80%)          | 0.748   |
| ≥60                             | 100 (59.17%)| 41 (57.75%)        | 59 (60.20%)          |         |
| Gender                          |            |                    |                      | 0.129   |
| Male                            | 136 (80.47%)| 61 (85.92%)        | 75 (76.53%)          |         |
| Female                          | 33 (19.53%)| 10 (14.08%)        | 23 (23.47%)          |         |
| BMI (kg/m²)                     |            |                    |                      | 0.013*  |
| <25                             | 118 (69.82%)| 54 (76.06%)        | 65 (66.33%)          |         |
| ≥25                             | 51 (30.18%)| 17 (23.94%)        | 33 (33.67%)          |         |
| Smoking history                 |            |                    |                      | 0.618   |
| Not heavy (No/light)            |            |                    |                      |         |
| Male                            | 136 (80.47%)| 61 (85.92%)        | 75 (76.53%)          |         |
| Female                          | 33 (19.53%)| 10 (14.08%)        | 23 (23.47%)          |         |
| Stage                           |            |                    |                      | 0.043*  |
| III/IIIB/IIIC                   | 33 (19.53%)| 19 (26.76%)        | 14 (14.29%)          |         |
| IV                              | 136 (80.47%)| 52 (73.24%)        | 84 (85.71%)          |         |
| Histology                       |            |                    |                      | 0.129   |
| Squamous carcinoma              |            |                    |                      |         |
| Male                            | 136 (80.47%)| 61 (85.92%)        | 75 (76.53%)          |         |
| Female                          | 33 (19.53%)| 10 (14.08%)        | 23 (23.47%)          |         |
| Adeno                           |            |                    |                      |         |
| Male                            | 111 (65.68%)| 41 (57.75%)        | 70 (71.43%)          |         |
| Female                          | 10 (5.92%)| 4 (5.63%)          | 6 (6.12%)            |         |
| Other                           |            |                    |                      |         |
| Male                            | 101 (59.76%)| 44 (61.97%)        | 57 (58.16%)          |         |
| Female                          | 33 (19.53%)| 10 (14.08%)        | 23 (23.47%)          |         |
| ECOG score                      |            |                    |                      | 0.059   |
| 0–1                             | 159 (94.08%)| 67 (94.37%)        | 92 (93.88%)          |         |
| 2–3                             | 10 (5.92%)| 4 (5.63%)          | 6 (6.12%)            |         |
| PD-L1 expression                |            |                    |                      | 0.731   |
| Unknown                         |            |                    |                      |         |
| Male                            | 118 (69.82%)| 54 (76.06%)        | 65 (66.33%)          |         |
| Female                          | 51 (30.18%)| 17 (23.94%)        | 33 (33.67%)          |         |
| Negative                        |            |                    |                      |         |
| Male                            | 68 (40.24%)| 27 (38.03%)        | 41 (41.84%)          |         |
| Female                          | 101 (59.76%)| 44 (61.97%)        | 57 (58.16%)          |         |
| Low (1–49%)                     |            |                    |                      |         |
| Male                            | 48 (28.40%)| 26 (36.62%)        | 22 (22.45%)          |         |
| Female                          | 10 (5.92%)| 4 (5.63%)          | 6 (6.12%)            |         |
| High (≥50%)                     |            |                    |                      |         |
| Male                            | 48 (28.40%)| 22 (30.99%)        | 26 (26.53%)          |         |
| Female                          | 10 (5.92%)| 4 (5.63%)          | 6 (6.12%)            |         |
| Treatment line of Immunotherapy |            |                    |                      | 0.593   |
| First                           | 51 (30.18%)| 23 (32.39%)        | 28 (28.57%)          |         |
| Non-first                       | 118 (69.82%)| 48 (67.61%)        | 70 (71.43%)          |         |
| Treatment regime                |            |                    |                      | 0.456   |
| Monotherapy                     |            |                    |                      |         |
| Male                            | 80 (47.34%)| 36 (50.70%)        | 44 (44.90%)          |         |
| Female                          | 89 (52.66%)| 35 (49.30%)        | 54 (55.10%)          |         |
| ICI+ chemotherapy               |            |                    |                      |         |
| Male                            | 80 (47.34%)| 36 (50.70%)        | 44 (44.90%)          |         |
| Female                          | 89 (52.66%)| 35 (49.30%)        | 54 (55.10%)          |         |
| Statin therapy                  |            |                    |                      | 0.250   |
| Male                            | 113 (66.86%)| 44 (61.97%)        | 69 (70.41%)          |         |
| Female                          | 56 (33.14%)| 27 (38.03%)        | 29 (29.59%)          |         |
| Baseline TG (mmol/L)            |            |                    |                      | 0.094   |
| <1.76                           |            |                    |                      |         |
| Male                            | 127 (75.15%)| 58 (81.69%)        | 69 (70.41%)          |         |
| Female                          | 42 (24.85%)| 13 (18.31%)        | 29 (29.59%)          |         |
| ≥1.76                           |            |                    |                      |         |
| Male                            | 130 (76.92%)| 55 (77.46%)        | 75 (76.53%)          |         |
| Female                          | 39 (33.14%)| 16 (22.54%)        | 23 (23.47%)          |         |
| Baseline LDL cholesterol(mmol/L)|            |                    |                      | 0.887   |
| <3.37                           |            |                    |                      |         |
| Male                            | 130 (76.92%)| 55 (77.46%)        | 75 (76.53%)          |         |
| Female                          | 39 (33.14%)| 16 (22.54%)        | 23 (23.47%)          |         |
| ≥3.37                           |            |                    |                      |         |

(Continued)
Table 1 (Continued).

| Characteristics            | All          | Hypcholesterolemia | Hypercholesterolemia | P-value |
|----------------------------|--------------|--------------------|----------------------|--------|
| Baseline HDL cholesterol(mmol/L) |              |                    |                      |        |
| ≥1.04                      | 129 (76.33%) | 56 (78.87%)        | 73 (74.49%)          | 0.508  |
| <1.04                      | 40 (23.67%)  | 15 (21.13%)        | 25 (25.51%)          |        |
| Response                   |              |                    |                      |        |
| PR                         | 43 (25.44%)  | 10 (14.08%)        | 33 (33.67%)          | 0.004* |
| SD                         | 54 (31.95%)  | 20 (28.17%)        | 34 (34.69%)          | 0.369  |
| PD                         | 72 (42.61%)  | 41 (57.75%)        | 31 (31.63%)          | <0.001*|
| ORR                        | 43 (25.44%)  | 10 (14.08%)        | 33 (33.67%)          | 0.004* |
| DCR                        | 97 (57.40%)  | 30 (42.25%)        | 67 (68.37%)          | 0.001* |

**Note:** *p<0.05.

**Abbreviations:** BMI, body mass index; ECOG score, Eastern Cooperative Oncology Group score; PD-L1, programmed cell death-1 ligand-1; ICIs, immune checkpoint inhibitors; TG, triglyceridemia; LDL, low-density lipoprotein; HDL, high-density lipoprotein; PR, partial response; SD, stable disease; PD, progression disease.

by logistic regression analysis. The Cox proportional hazards model was used to calculate the hazard ratio (HR) and corresponding 95% confidence interval (CI). Receiver-operating characteristic (ROC) curves were used to estimate the sensitivity and specificity of biomarkers by calculating the area under the curve (AUC). Statistical significance was indicated by a two-sided P value <0.05.

**Results**

**Patients Characteristics**

A total of 193 patients with advanced NSCLC who received ICI-based treatment at Ningbo Medical Treatment Center, Li Huili Hospital from August 1, 2017 to December 31, 2019 were screened, and 169 patients (87.6%) were enrolled. At the time of data collection, 72 patients had experienced progression (42.60%) and 98 patients (57.99%) were had died. The baseline characteristics of the patients with hypcholesterolemia or hypercholesterolemia and efficacous outcomes of ICI-based treatment, are summarized in Table 1. Patients with hypercholesterolemia were more likely in stage IV (*P=0.043) or with BMI≥25kg/m² (*P=0.013), and had a better response to ICI-based treatment according to the higher rate of ORR (*P=0.004) and DCR (*P=0.001).

Patients were divided into two groups based on PD-L1 expression. In the Unknown/no/low-PD-L1 group (n=121), patients with hypercholesterolemia had higher baseline TG (*P=0.041) or with BMI≥25kg/m² (*P=0.027) and a better response to ICI-based treatment as judged by their higher rate of PR (*P=0.001) and the lower rate of PD (*P=0.001, Table 2). Although in the high-PD-L1 group, patients with hypercholesterolemia had lower ECOG (Eastern Cooperative Oncology Group) scores (P=0.050), there was no significant difference in their response to ICI-based treatment compared with patients with hypcholesterolemia (Table 3).

**Objective Response to ICI-Based Treatment**

Patients with hypercholesterolemia had a higher objective response rate (ORR) (33.67%) and disease control rate (DCR) (68.37%) than those with hypcholesterolemia (ORR: 14.08%, *P=0.004; DCR: 42.25%, *P=0.001 Figure 1A). And in the unknown/no/low PD-L1 group, the patients with hypercholesterolemia also responded more favorably to ICI-based treatment. The ORR of patients with hypercholesterolemia was 30.56%, while it was 6.12% in patients with hypcholesterolemia (P<0.001). The DCR was 62.50% vs 32.65% (P=0.002) for the same two groups, respectively (Figure 1B). In the high-PD-L1 group, the response was similar (ORR: 42.31% vs 31.82%, P=0.454; DCR: 84.62% vs 63.64, P=0.094, Figure 1C).

**Survival Analysis**

The median PFS times of patients with hypercholesterolemia and hypcholesterolemia were 7.9 months vs 4.4 months, respectively (95% CI: 4.620–7.380, *P<0.001, Figure 2A). And the median OS times in the two groups were 11 months and 8 months, respectively (95% CIs of 8.980–10.420, *P<0.001, Figure 2B). In the unknown/no/low PD-L1 group, the median PFS of the patients with hypercholesterolemia was 7.5 months, which was higher than that of patients with hypcholesterolemia (3.5 months, 95% CI: 4.477–5.923,
Table 2 Baseline Characteristics of Patients in Unknown/No/Low PD-L1 Group (n=121)

| Characteristics                  | All          | Hypcholesterolemia | Hypercholesterolemia | P-value |
|----------------------------------|--------------|--------------------|----------------------|---------|
| Age (years)                      |              |                    |                      |         |
| <60                              | 43 (35.54%)  | 18 (36.73%)        | 25 (34.72%)          | 0.820   |
| ≥60                              | 78 (64.46%)  | 31 (63.27%)        | 47 (65.28%)          |         |
| Gender                           |              |                    |                      |         |
| Male                             | 99 (58.58%)  | 44 (89.80%)        | 55 (76.39%)          | 0.061   |
| Female                           | 22 (41.42%)  | 5 (10.20%)         | 17 (23.61%)          |         |
| BMI (kg/m²)                      |              |                    |                      |         |
| <25                              | 92 (76.03%)  | 39 (79.59%)        | 53 (73.61%)          | 0.027*  |
| ≥25                              | 29 (23.97%)  | 10 (20.41%)        | 19 (26.39%)          |         |
| Smoking history                  |              |                    |                      |         |
| Not heavy (No/light)             | 48 (39.67%)  | 20 (40.82%)        | 28 (38.89%)          | 0.832   |
| Heavy                            | 73 (60.33%)  | 29 (59.18%)        | 44 (61.11%)          |         |
| Stage                            |              |                    |                      | 0.373   |
| III/IIIB/IIIIC                   | 18 (14.88%)  | 9 (18.37%)         | 9 (12.50%)           |         |
| IV                               | 103 (85.12%) | 40 (81.63%)        | 63 (87.50%)          |         |
| Histology                        |              |                    |                      |         |
| Squamous carcinoma               | 34 (28.10%)  | 19 (38.78%)        | 15 (20.83%)          | 0.096   |
| Adeno                            | 79 (65.29%)  | 27 (55.10%)        | 52 (72.22%)          |         |
| Other                            | 8 (6.61%)    | 3 (6.12%)          | 5 (6.94%)            |         |
| ECOG score                       |              |                    |                      | 0.340   |
| 0–1                              | 116 (95.87%) | 48 (97.96%)        | 68 (94.44%)          |         |
| 2–3                              | 5 (4.13%)    | 1 (2.04%)          | 4 (5.56%)            |         |
| Statin therapy                   |              |                    |                      | 0.520   |
| No                               | 83 (68.60%)  | 32 (65.31%)        | 51 (70.83%)          |         |
| Yes                              | 38 (31.40%)  | 17 (34.69%)        | 21 (29.17%)          |         |
| Treatment line of Immunotherapy  |              |                    |                      | 0.736   |
| First                            | 35 (28.93%)  | 15 (30.61%)        | 20 (27.78%)          |         |
| Non-first                        | 86 (71.07%)  | 34 (69.39%)        | 52 (72.22%)          |         |
| Treatment regime                 |              |                    |                      | 0.358   |
| Monotherapy                      | 34 (28.10%)  | 16 (32.65%)        | 18 (25.00%)          |         |
| ICIs+ chemotherapy               | 87 (71.90%)  | 33 (67.35%)        | 54 (75.00%)          |         |
| Baseline TG (mmol/L)             |              |                    |                      |         |
| <1.76                            | 95 (78.51%)  | 43 (87.76%)        | 52 (72.22%)          | 0.041*  |
| ≥1.76                            | 26 (21.49%)  | 6 (12.24%)         | 20 (27.78%)          |         |
| Baseline LDL cholesterol (mmol/L)|              |                    |                      | 0.491   |
| <3.37                            | 95 (78.51%)  | 40 (81.63%)        | 55 (76.39%)          |         |
| ≥3.37                            | 26 (21.49%)  | 9 (18.37%)         | 17 (23.61%)          |         |
| Baseline HDL cholesterol (mmol/L)|              |                    |                      | 0.678   |
| ≥1.04                            | 94 (77.69%)  | 39 (79.59%)        | 55 (76.39%)          |         |
| <1.04                            | 27 (22.31%)  | 10 (20.41%)        | 17 (23.61%)          |         |
| Response                         |              |                    |                      |         |
| PR                               | 25 (20.66%)  | 3 (6.12%)          | 22 (30.56%)          | 0.001*  |
| SD                               | 36 (29.75%)  | 13 (26.53%)        | 23 (31.94%)          | 0.523   |
| PD                               | 60 (49.59%)  | 33 (67.35%)        | 27 (37.50%)          | 0.001*  |
| ORR                              | 25 (20.66%)  | 3 (6.12%)          | 22 (30.56%)          | <0.001* |
| DCR                              | 61 (50.41%)  | 16 (32.65%)        | 45 (62.50%)          | 0.002*  |

Note: *p<0.05.

Abbreviations: BMI, body mass index; ECOG score, Eastern Cooperative Oncology Group score; PD-L1, programmed cell death-1 ligand-1; ICIs, immune checkpoint inhibitors; TG, triglyceridemia; LDL, low-density lipoprotein; HDL, high-density lipoprotein; PR, partial response; SD, stable disease; PD, progression disease.
Table 3 Baseline Characteristics of Patients in High PD-L1 Group (n=48)

| Characteristics          | All    | Hypcholesterolemia | Hypercholesterolemia | P-value |
|-------------------------|--------|--------------------|----------------------|---------|
| Age(years)              |        |                    |                      |         |
| <60                     | 26 (54.17%) | 12 (54.55%)         | 14 (53.85%)          | 0.961   |
| ≥60                     | 22 (45.83%) | 10 (45.45%)         | 12 (46.15%)          |         |
| Gender                  |        |                    |                      | 0.977   |
| Male                    | 37 (77.08%) | 17 (77.27%)         | 20 (76.92%)          |         |
| Female                  | 11 (22.92%) | 5 (22.73%)          | 6 (23.08%)           |         |
| BMI (kg/m²)             |        |                    |                      | 0.202   |
| <25                     | 26 (54.17%) | 14 (63.64%)         | 12 (46.15%)          |         |
| ≥25                     | 22 (45.83%) | 8 (36.36%)          | 14 (53.85%)          |         |
| Smoking history         |        |                    |                      | 0.203   |
| Not heavy (No/light)    | 20 (41.67%) | 7 (31.82%)          | 13 (50.00%)          |         |
| Heavy                   | 28 (58.33%) | 15 (68.18%)         | 13 (50.00%)          |         |
| Stage                   |        |                    |                      | 0.051   |
| III/IIIB/IIIC           | 15 (31.25%) | 10 (45.45%)         | 5 (19.23%)           |         |
| IV                      | 33 (68.75%) | 12 (54.55%)         | 21 (80.77%)          |         |
| Histology               |        |                    |                      | 0.920   |
| Squamous carcinoma      | 14 (29.17%) | 7 (31.82%)          | 7 (26.92%)           |         |
| Adeno                   | 32 (66.67%) | 14 (63.64%)         | 18 (69.23%)          |         |
| Other                   | 2 (4.17%) | 1 (4.55%)          | 1 (3.85%)            |         |
| ECOG score              |        |                    |                      | 0.050*  |
| 0–1                     | 43 (89.58%) | 19 (86.36%)         | 24 (92.31%)          |         |
| 2–3                     | 5 (10.42%) | 3 (13.64%)          | 2 (7.69%)            |         |
| Statin therapy          |        |                    |                      | 0.295   |
| No                      | 30 (62.5%) | 12 (54.55%)         | 18 (69.23%)          |         |
| Yes                     | 18 (37.5%) | 10 (45.45%)         | 8 (30.77%)           |         |
| Treatment line of Immunotherapy | | | | |
| First                   | 16 (33.33%) | 8 (36.36%)          | 8 (30.77%)          | 0.682   |
| Non-first               | 32 (66.67%) | 14 (63.64%)         | 18 (69.23%)          |         |
| Treatment regime        |        |                    |                      | 0.116   |
| Monotherapy             | 46 (95.83%) | 20 (90.91%)         | 26 (100.00%)         |         |
| ICIs+ chemotherapy      | 4 (8.17%) | 2 (9.09%)           | 0 (0.00%)            |         |
| Baseline TG (mmol/L)    |        |                    |                      | 0.838   |
| <1.76                   | 32 (66.67%) | 15 (68.18%)         | 17 (65.38%)          |         |
| ≥1.76                   | 16 (33.33%) | 7 (31.82%)          | 9 (34.62%)           |         |
| Baseline LDL cholesterol(mmol/L) | | | | |
| <3.7                    | 35 (72.92%) | 15 (68.18%)         | 20 (76.92%)          | 0.497   |
| ≥3.7                    | 13 (27.08%) | 7 (31.82%)          | 6 (23.08%)           |         |
| Baseline HDL cholesterol(mmol/L) | | | | |
| ≥1.04                   | 35 (72.92%) | 17 (77.27%)         | 18 (69.23%)          | 0.532   |
| <1.04                   | 13 (27.08%) | 5 (22.73%)          | 8 (30.77%)           |         |
| Response                |        |                    |                      |         |
| PR                      | 18 (37.50%) | 7 (31.82%)          | 11 (42.31%)          | 0.454   |
| SD                      | 18 (37.50%) | 7 (31.82%)          | 11 (42.31%)          | 0.454   |
| PD                      | 12 (25.00%) | 8 (36.36%)          | 4 (15.38%)           | 0.094   |
| ORR                     | 18 (37.50%) | 7 (31.82%)          | 11 (42.31%)          | 0.454   |
| DCR                     | 36 (75.00%) | 14 (63.64%)         | 22 (84.62%)          | 0.094   |

Note: *p<0.05.

Abbreviations: BMI, body mass index; ECOG score, Eastern Cooperative Oncology Group score; PD-L1, programmed cell death-1 ligand-1; ICIs, immune checkpoint inhibitors; TG, triglyceridemia; LDL, low-density lipoprotein; HDL, high-density lipoprotein; PR, partial response; SD, stable disease; PD, progression disease.
expression cohort, BMI<25kg/m and olemia P<0.001, -1–12.089, -). C elypercholesterol eatment. ypocholesterolemia A - months, than high-PD-L1 7.751–9.249, the median =0.599). Furthermore, TC≥5.18mmol/L, TG≥1.76mmol/L, HDL cholesterol≥1.04 and BMI≥25kg/m² resulted in more objective response compared with TC<5.18mmol/L, TG<1.76mmol/ L, HDL cholesterol<1.04 and BMI<25kg/m² (P=0.005, HR =3.097; P=0.032, HR =2.279; P=0.006, HR =2.928; P=0.000, HR =9.792). Based on multivariate analysis, age, BMI≥25kg/m², stage, statin therapy, baseline hypercholesterolemia and HDL cholesterol were associated with objective response (Table 6).

Predictive Value of Baseline Cholesterol Level
In the ROC curve for disease control in the total population, the AUC for baseline cholesterol level was 0.706 (p<0.001) based on a 5.265mmol/L cut-off, While the AUC was 0.643 (p=0.001) for PD-L1 expression (Figure 3), thus indicating lower sensitivity and specificity in the total population.

Discussion
Cholesterol has been reported to substantially contribute to progression of several types of cancer. ICIs have
Figure 2 Survival analysis of patients with hypercholesterolemia or hypocholesterolemia based on the ICI-based treatment.

Notes: PFS and OS comparison of patients with different level of serum cholesterol; (A) PFS in total population (n=169, mPFS:4.4m VS 7.9m, P<0.001); (B) OS in total population (n=169, mOS:8.0m VS 11.0m, P<0.001); (C) PFS in population of unknown/no/low PD-L1 expression (n=121, mPFS:3.7m VS 7.5m, P<0.001); (D) OS in population of unknown/no/low PD-L1 expression (n=121, mPFS:7.2m VS 10.0m, P=0.001); (E) PFS in population of high PD-L1 expression (n=48, mPFS:4.9m VS 8.9m, P<0.001); (F) OS in population of PD-L1 expression (n=48, mPFS:9.9m VS 13.9m, P<0.001). PFS, progression-free survival; OS, overall survival.
frequently been used to treat NSCLC in recent years. In this study, we found that baseline hypercholesterolemia was associated with a positive response to ICI-based treatment and with longer PFS and OS in advanced NSCLC patients. The level of serum cholesterol is therefore a potential marker for prediction of efficacy and survival of patients with advanced NSCLC undergoing ICI-based therapy.

Cholesterol plays an important role in the metabolism and growth of every type of mammalian cell, stabilizes cell membranes, and is a precursor of vitamins and hormones. Recently, cholesterol has gained a lot of attention for its role in cancer and its response to treatment.

Table 4 Univariate and Multivariate Analyses of Clinical Parameters of PFS in Overall Patients

| Factors                        | Univariate Analysis | Multivariate Analysis |
|--------------------------------|---------------------|-----------------------|
|                                | HR                  | 95% CI                | P-value | HR                  | 95% CI                | P-value |
| Age(years)                     |                     |                       |         |                     |                       |         |
| <60/≥60                         | 6.226               | 2.978–13.016          | 0.000*  | 1.068               | 1.036–1.102          | 0.000*  |
| Gender                         |                     |                       |         |                     |                       |         |
| Male/Female                    | 0.845               | 0.454–1.574           | 0.595   |                     |                       |         |
| BMI (kg/m2) <25/≥25            | 0.704               | 4.620–7.380           | 0.000*  | 0.902               | 0.803–1.014          | 0.084   |
| Smoking history Not heavy (No/light)/Heavy | 1.147               | 0.703–1.871           | 0.584   |                     |                       |         |
| Stage III (IIIB, IIIC)/IV      | 2.998               | 1.207–7.447           | 0.018*  | 2.360               | 0.793–7.029          | 0.123   |
| Histology Squamous carcinoma/Adeno | 0.824               | 0.483–1.408           | 0.479   |                     |                       |         |
| Squamous carcinoma/Other       | 0.779               | 0.264–2.296           | 0.650   |                     |                       |         |
| ECOG score 0–1/2–3             | 0.781               | 0.283–2.150           | 0.632   |                     |                       |         |
| PD-L1 expression Not high (unknown/no/low)/high | 0.394               | 0.214–0.726           | 0.003*  | 0.763               | 0.613–0.951          | 0.016*  |
| Statin therapy No/Yes          | 0.370               | 0.199–0.691           | 0.002*  | 0.448               | 0.234–0.859          | 0.016*  |
| Treatment line of Immunotherapy First/Non-first | 1.021               | 0.602–1.732           | 0.938   | 0.838               | 0.468–1.500          | 0.551   |
| Treatment regime Monotherapy/ICIs+ chemotherapy | 1.011               | 0.631–1.620           | 0.964   |                     |                       |         |
| Baseline TC (mmol/L) <5.18/≥5.18 | 0.268               | 0.161–0.447           | 0.000*  | 0.231               | 0.135–0.395          | 0.000*  |
| Baseline TG (mmol/L) <1.76/≥1.76 | 0.630               | 0.349–1.137           | 0.125   | 0.890               | 0.404–1.961          | 0.773   |
| Baseline LDL cholesterol(mmol/L) <3.37/≥3.37 | 0.786               | 0.443–1.397           | 0.412   |                     |                       |         |
| Baseline HDL cholesterol(mmol/L) ≥1.04/<1.04 | 0.568               | 0.304–1.059           | 0.075   | 2.146               | 0.812–5.673          | 0.124   |

Note: *p<0.05.

Abbreviations: BMI, body mass index; ECOG score, Eastern Cooperative Oncology Group score; PD-L1, programmed cell death-1 ligand-1; ICIs, immune checkpoint inhibitors; TC, total cholesterol; TG, triglyceridemia; LDL, low-density lipoprotein; HDL, high-density lipoprotein; PR, partial response; SD, stable disease; PD, progression disease.
### Table 5 Univariate and Multivariate Analyses of Clinical Parameters of OS in Overall Patients

| Factors                              | Univariate Analysis | Multivariate Analysis |
|--------------------------------------|---------------------|-----------------------|
|                                      | HR                  | 95% CI                | P-value | HR                  | 95% CI                | P-value |
| Age(years)                           |                     |                       |         |                     |                       |         |
| <60/≥60                              | 1.055               | 0.655–1.698           | 0.827   |                     |                       |         |
| Gender                               |                     |                       |         |                     |                       |         |
| Male/Female                          | 1.313               | 0.872–1.977           | 0.193   | 1.191               | 0.699–2.030           | 0.520   |
| BMI (kg/m2)                          | 0.368               | 8.980–10.420          | 0.019*  | 0.990               | 0.904–1.085           | 0.837   |
| Smoking history                      |                     |                       |         |                     |                       |         |
| Not heavy (No/light)/Heavy           | 0.876               | 0.540–1.420           | 0.591   |                     |                       |         |
| Stage                                |                     |                       |         |                     |                       |         |
| III(IIIIII/IV)                       | 1.660               | 1.166–2.3627          | 0.005*  | 1.300               | 0.724–2.336           | 0.380   |
| Histology                            | 0.664               | 0.250–1.666           | 0.366   | 1.606               | 0.605–4.264           | 0.342   |
| ECOG score                           | 0.736–3.164         | 0.256                 |         |                     |                       |         |
| PD-L1 expression                     |                     |                       |         |                     |                       |         |
| Not high (unknown/no/low)/high       | 0.450               | 0.285–0.711           | 0.000*  | 0.786               | 0.650–0.950           | 0.013*  |
| Statin therapy                       |                     |                       |         |                     |                       |         |
| No/Yes                               | 1.0123              | 0.661–1.550           | 0.955   |                     |                       |         |
| Treatment line of Immunotherapy     |                     |                       |         |                     |                       |         |
| First/Non-first                      | 1.040               | 0.663–1.630           | 0.865   |                     |                       |         |
| Treatment regime                     | 1.478               | 0.985–2.217           | 0.050*  | 1.535               | 0.963–2.446           | 0.072   |
| Baseline TC (mmol/L)                 |                     |                       |         |                     |                       |         |
| ≤5.18/≥5.18                          | 0.295               | 0.190–0.458           | 0.000*  | 0.235               | 0.147–0.377           | 0.000*  |
| Baseline TG (mmol/L)                 |                     |                       |         |                     |                       |         |
| <1.76/≤1.76                          | 0.599               | 0.369–0.972           | 0.038*  | 0.909               | 0.494–1.674           | 0.760   |
| Baseline LDL cholesterol (mmol/L)    |                     |                       |         |                     |                       |         |
| <3.37/≥3.37                          | 0.629               | 0.377–1.050           | 0.076   | 0.882               | 0.540–1.442           | 0.616   |
| Baseline HDL cholesterol (mmol/L)    |                     |                       |         |                     |                       |         |
| <1.04/≥1.04                          | 0.764               | 0.471–1.240           | 0.275   |                     |                       |         |

**Note:** *p<0.05.

**Abbreviations:** BMI, body mass index; ECOG score, Eastern Cooperative Oncology Group score; PD-L1, programmed cell death-1 ligand-1; ICIs, immune checkpoint inhibitors; TC, total cholesterol; TG, triglycerides; LDL, low-density lipoprotein; HDL, high-density lipoprotein; PR, partial response; SD, stable disease; PD, progression disease.

Association with cancer. Earlier research indicated that cholesterol has a significant role in initiating and promoting some types of cancer.23–25 Meanwhile, the relationship between cholesterol and immune cells has also frequently been studied. On the one hand, some research has shown that in T cells, the biosynthesis of cholesterol is highly upregulated by liver X receptor (LXR) inactivation, which activates T cells.26,27 This was related to cholesterol maintaining the rigidity of immune cell membranes and transmitting cellular signals by receptor co-localization, thus demonstrating the positive role of cholesterol in the immune response.28 On the other hand, other studies showed that for tumor-infiltrating T cells,
accumulation of cholesterol induced the expression of immune checkpoints PD-1 and 2B4, leading to T cell exhaustion. Reducing cholesterol may enhance T cell-based immunotherapy. However, we suspected increasing expression of PD-1 on the surface of tumor-infiltrating T cells may in some cases increase PD-L1 binding and the response to ICI-based treatment. In addition to T cells, it was also observed that cholesterol in NK cells was associated with activation of immune signaling. Cholesterol influx into DC cells has been reported to enhance antigen presentation.

In our study, we found the level of serum cholesterol to be significantly associated with better responses to ICI-
observed that statin use led to longer PFS and better ORR in the cohort, consistent with earlier research.\textsuperscript{15}

However, our study has some limitations. First, the retrospective design and small sample size may not get a definitive conclusion, and second, we did not analyze outcomes using different ICI drugs. Therefore, larger prospective studies will be required to validate the present results, and studies using other ICI drugs will be needed to test whether our results represent a generalized response.

**Conclusion**

In conclusion, the present study indicated that baseline serum cholesterol level was associated with clinical benefit for advanced NSCLC patients who undergo ICI-based treatment. And it could be a promising prognostic predictor for ICI-based treatment in NSCLC.

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**Disclosure**

The authors report no conflicts of interest in this work.

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